Chloroquine: Physiological Basis of Drug Resistance in Plasmodium berghei

Abstract. *Mouse erythrocytes, parasitized by chloroquine-sensitive plasmodia, concentrate this drug in vivo to levels over twice as high as erythrocytes parasitized by chloroquine-resistant plasmodia; nonparasitized red cells accumulate little chloroquine. Selective toxicity of this drug may depend upon a special drug-concentrating mechanism of plasmodia, while resistance may result from an impairment of such mechanism.*

Emergence of strains of *Plasmodium falciparum* that are resistant to the antimalarial drug chloroquine (CQ) prompted our study of the nature of CQ resistance in *Plasmodium berghei*. This drug inhibits nucleic acid syntheses in *P. berghei* (*1*) as well as in other cells (*2*). Conclusions from other studies (*3*) are that such inhibitions of DNA replication and RNA transcription result from formation of a molecular complex of native DNA with CQ (*4*) and that these inhibitions are responsible for the antimicrobial effect of the drug. Susceptibility or resistance to CQ cannot be explained on the basis of structural or compositional differences between the DNA's of susceptible or resistant cells but may depend upon differences in capabilities of such cells to permit passage or accumulation of critical concentrations of the drug (*3*). Our study has tested this hypothesis by comparing the concentrations of CQ, after administration of the drug to mice, in normal erythrocytes with those parasitized either by CQ-sensitive or CQ-resistant *P. berghei*. Our results are consistent with our working hypothesis.

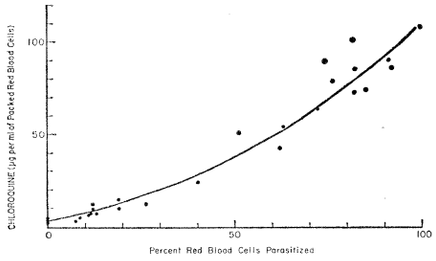

Fig. 1. Correlation between parasitemia and uptake of chloroquine by erythrocytes. Mice, 2 to 4 days after inoculation with CQ-sensitive *P. berghei*, and uninfected mice were given intraperitoneal injections of 40 mg/kg of ^{14}C-labeled CQ (0.35 μc/mg; New England Nuclear). After 4 hours, the mice were anesthetized and were bled by axillary artery section. Red cells and plasma of oxalated blood were separated by centrifugation, added to enough 5*N* NaOH to insure a *p*H greater than 12, and extracted with ethyl ether. Ether was evaporated, and the solids were dissolved in a dioxane scintillation fluid and counted in a Nuclear-Chicago liquid scintillation counter.

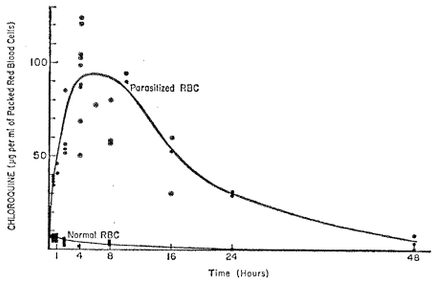

Fig. 2. Time course of chloroquine concentration by parasitized and nonparasitized erythrocytes. Experimental techniques were the same as for Fig. 1.

Male white mice (strain ICR) were infected by intraperitoneal inoculation of 10^8 mouse erythrocytes parasitized either by the CQ-sensitive, NYU-2 strain of *P. berghei* (*5*), or by a derivative of this strain that we had bred selectively for CQ resistance by 17 months of continuous mouse passages in the presence of increasing concentrations of the drug. These plasmodia were completely resistant to the chemotherapeutic action of daily, parenterally administered doses (per kilogram of body weight) of 40 mg of CQ, 63 mg of mepacrine, and 130 mg of quinine for 5 days. Chloroquine, labeled with ^{14}C in the quinoline ring, was administered intraperitoneally; the animals were killed, the parasites were counted, and radiochemical analyses of the CQ content of blood and solid tissues were made.

Quantities of CQ associated with erythrocytes were proportional to fractions of such cells parasitized by CQ-susceptible *P. berghei* (Fig. 1). We attribute the deviation of this correlation from linearity to our observations that at later stages of the infection (that is, at higher levels of parasitemia) many red cells contained more than one parasite and that the parasites themselves were on the average larger. Parasitized erythrocytes contained 25 times more CQ than normal erythrocytes did; concentration of the drug in completely parasitized populations of red cells attained 100 μg/ml of packed cells.

Figure 2 depicts the time course of accumulation of CQ in both parasitized and nonparasitized erythrocytes after intraperitoneal administration of CQ. Concentration of the drug in parasitized cells attained a maximum after approximately 4 hours and then declined, while the slight uptake of drug by normal erythrocytes, as well as the uptake by plasma, liver, and spleen (not shown), was maximum less than 1 hour after injection of CQ. In 4-hour samples, concentration of CQ per unit volume of packed parasitized erythrocytes was 100 times higher than in the same volume of plasma in which red cells had been suspended and sedimented. This difference, as well as the fact that the concentration of CQ in parasitized erythrocytes was continuing

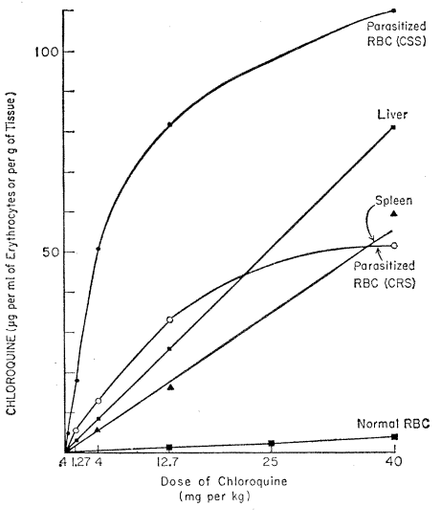

Fig. 3. Correlation between the uptake of chloroquine by blood and tissues and the dosage of chloroquine. Experimental techniques were the same as for Fig. 1. Liver and spleen were completely digested in NaOH before ether extraction. All values are the means of three or more experiments. Values for parasitized erythrocytes are arithmetically derived. We assume that the nonparasitized cells of an infected animal contain the same amount of chloroquine as the red cells from a normal animal that received the same dose of drug. The amount of chloroquine expected in the nonparasitized fraction of the blood sample is substracted from the total found, and the remainder is attributed to that fraction of cells known to be parasitized. *CSS*, chloroquine-sensitive strain; *CRS*, chloroquine-resistant strain.

to rise while its concentration in plasma was declining, suggests existence of a drug-concentrating mechanism in plasmodia or in parasitized red cells in contrast to an exchange of CQ by free diffusion; only insignificant quantities of CQ were removed by washing parasitized erythrocytes in 0.15*M* NaCl.

A marked difference between concentrations of CQ accumulated in red cells parasitized by either susceptible or resistant *P. berghei* is shown in Fig. 3, in which these drug levels are represented as a function of the quantities of drug administered. Depending upon these quantities, concentrations of CQ were two to three times higher in red cells containing sensitive plasmodia than in those containing CQ-resistant *P. berghei*. Difference in the content of CQ per parasite is even greater, since erythrocytes parasitized with resistant *P. berghei* contain an average of 1.6 parasites to every erythrocytic CQ-sensitive parasite. Even at the nearly toxic dose of 40 mg/kg, the concentration of CQ attained with resistant *P. berghei* was no more than that attained with sensitive parasites upon administration of only 4 mg/kg. The greater accumulation of CQ in erythrocytes parasitized by sensitive *P. berghei* in comparison to lower levels attained in normal red cells, liver, and spleen explains the selective toxicity of CQ, upon which its usefulness as a chemotherapeutic drug is based. Furthermore, a linear relation exists between the degree of accumulation of CQ in tissues and normal red cells and the administered dose. In contrast, the uptake response of parasitized erythrocytes to graded doses of the drug more nearly resembles a correlation in which the logarithms of doses are proportional to the probits of the CQ concentrations attained. Such a specific accumulation of the drug in the parasite–red cell system may be ascribed to a mechanism that could be based upon the following conditions: (i) active transport of CQ, or (ii) free diffusion of CQ followed by reversible binding to intracellular sites.

Our experiments do not distinguish between these two mechanistic explanations; they also do not distinguish whether such a mechanism is intrinsic to the plasmodia or to the whole parasitized erythrocyte. The results do, however, suggest that CQ is selectively toxic because it attains higher concentrations in parasitized cells than in normal tissue cells, and that plasmodial resistance to CQ is based on an impairment of the mechanism by which such drug levels are accumulated.

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References and Notes

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Protective Action of Polycyclic Hydrocarbons against Induction of Adrenal Necrosis by Dimethylbenzanthracene

Abstract. *Treatment of rats with certain polycyclic hydrocarbons shifts the hydroxylation of dimethylbenzanthracene (DMBA) by liver microsomes from the side-chain to the ring. Protection by these hydrocarbons against dimethylbenzanthracene-induced adrenal necrosis is possibly achieved by decreasing the yield of the 7-hydroxymethyl derivative of dimethylbenzanthracene which may act as the necrotic agent by virtue of its structural resemblance to the adrenocortical steroids.*

The classical experiments of Huggins (*1*) and Dao (*2*) show that the administration of certain aromatic substances to rats prior to the feeding of 7,12-dimethylbenz(*a*)anthracene (DMBA) completely inhibits the induction of adrenal necrosis by DMBA and also gives some protection against the development of mammary cancer. Also the administration of a small dose of polycyclic hydrocarbon to rats induces a marked increase in hydroxylating enzymes in the liver (*4*). It has been shown that the gastrointestinal tract, lungs, and kidneys are also sites where hydroxylating enzymes increase after injection of polycyclic hydrocarbons (*5*). Boyland and Sims (*6*) have found that DMBA is metabolized in the rat mainly by oxidation of the methyl groups to the isomeric monohydroxymethyl derivatives, in contrast to phenanthrene, benz(*a*)anthracene, dibenz(*a,h*)anthracene and other unsubstituted aromatic hydrocarbons which are oxidized at reactive double bonds in the nucleus.

Several mechanisms have been pro-

Table 1. Effect of prior treatment with polycyclic hydrocarbons on the formation of ^{14}C-DMBA metabolites by rat liver microsomes. The polycyclic hydrocarbon (10 mg) dissolved in oil was given orally to rats (50 to 60 days of age), and the animals were killed 2 days later. The microsomes (8000*g* supernatant) from 50 mg of liver were incubated under oxygen for 1 hour at 37°C with DMBA-12-^{14}C (1.5×10^5 count/min in 3 μg), nicotinamide adenine dinucleotide phosphate (0.3 m*M*) and glucose-6-phosphate (3 m*M*) in 4 ml of phosphate buffer (0.1*M*), *p*H 7.4. The amount of radioactivity remaining in the aqueous medium after extraction with ether at *p*H 1 was then determined. The ether-soluble metabolites were separated by thin-layer chromatography and located by autoradiography.

Hydrocarbon	^{14}C in aqueous medium after extraction with ether (%)	Expts. (No.)	Ether-soluble fraction after chromatography		
			Unchanged DMBA (10^3 count/min)	Hydroxymethyl—MBA region (10^3 count/min)	Polar products near origin (10^3 count/min)
None (oil only)	27.1 ± 4.3	8	2.1	3.0	1.5
3-Methylcholanthrene	56.9 ± 8.4	8	1.0	0.6	2.7
Dibenz(*a,h*)anthracene	48.5 ± 9.0	5	1.3	.7	3.7
Naphthalene*	24.7 30.0	2	2.3	1.7	1.5

* Does not protect against adrenal necrosis (*2*).