The Antimalarial Drug Mefloquine Binds to Membrane Phospholipids†

REKHA CHEVLI AND COY D. FITCH*

Department of Internal Medicine, Saint Louis University School of Medicine, St. Louis, Missouri 63104

Received 30 November 1981/Accepted 25 January 1982

The new antimalarial drug mefloquine bound with high affinity \((K_d = 3 \times 10^{-7} \text{ M})\) to membrane lipids of normal mouse erythrocytes and of erythrocytes infected either with chloroquine-susceptible or chloroquine-resistant \(Plasmodium berghei\). Approximately 80 nmol of mefloquine was bound per mg of total lipid. Mefloquine also bound to purified phospholipids with high affinity \((K_d = 3 \times 10^{-7} \text{ M})\). Phosphatidylinositol and phosphatidylserine bound 300 to 400 nmol of mefloquine per mg. Phosphatidylcholine and phosphatidylethanolamine bound approximately 100 nmol of mefloquine per mg. Mefloquine did not bind to hemoglobin with high affinity, but it bound to free ferriprotoporphyrin IX with a \(K_d\) of \(\sim 3 \times 10^{-7} \text{ M}\). In comparison with mefloquine, chloroquine did not bind effectively to purified phospholipids, although it is known to bind with high affinity to free ferriprotoporphyrin IX. Greater binding to phospholipids may account for the superiority of mefloquine in the treatment of chloroquine-resistant malaria.

Mefloquine [WR 142,490; \(\alpha\)-(2-piperidyl)-2,8-bis(trifluoromethyl)-4-quinolinemethan] is a new antimalarial drug (18) that is effective in the treatment of chloroquine-resistant malaria (21). It is a quinoline derivative, as is chloroquine, but it is more hydrophobic than chloroquine. Because both are quinoline derivatives, the possibility has been considered that the chemotherapeutic effects of mefloquine and chloroquine are mediated through identical receptors (8). Consistent with this possibility, mefloquine is a competitive inhibitor of chloroquine binding in erythrocytes infected with \(Plasmodium berghei\) (7), and chloroquine is a competitive inhibitor of mefloquine binding (8). Against the possibility of identical receptors are the following observations. The capacity of infected and uninfected erythrocytes to bind mefloquine with high affinity greatly exceeds the capacity to bind chloroquine with high affinity (6, 8). The apparent \(K_d\) of chloroquine as a competitive inhibitor of mefloquine binding is several orders of magnitude higher than the apparent \(K_d\) for chloroquine binding to its receptor, \(10^{-3} \text{ M}\) versus \(10^{-8} \text{ M}\) (6, 8). Erythrocytes infected with chloroquine-resistant malaria parasites accumulate less chloroquine than erythrocytes infected with chloroquine-susceptible malaria parasites (6, 11), whereas chloroquine resistance has no appreciable effect on mefloquine accumulation (8). Also, the process of chloroquine accumulation by erythrocytes infected with chloroquine-susceptible \(P. berghei\) requires energy (11), whereas the accumulation of mefloquine apparently does not (8). These observations could be explained if mefloquine has greater access than chloroquine to identical receptors (8), but they also could be explained by the existence of a second receptor for mefloquine. The high-affinity receptor for chloroquine in erythrocytes infected with malaria parasites is ferriprotoporphyrin IX (FP) (3, 9, 19). We now present evidence that mefloquine interacts strongly both with FP and with certain phospholipids.

MATERIALS AND METHODS

Male Swiss-Webster mice weighing approximately 20 g were purchased from Hilltop Laboratories for use as donors of erythrocytes. For some of the experiments, groups of mice were infected either with chloroquine-resistant or with chloroquine-susceptible \(P. berghei\) of the NYU-2 strain. These two lines of parasites have been described in detail previously (6, 11). To prepare membranes, heparinized blood was pooled from groups of mice and washed twice (6, 10) with an isotonic medium (10) buffered to pH 7.4 with 50 mM phosphate (standard medium). As much as possible of theuffy coat was discarded with each wash. After washing, a 50% suspension of erythrocytes in the standard medium was cooled to 4°C and lysed by mixing with 20 volumes of cold, distilled water buffered to pH 8 with 5 mM phosphate. The hemolysate then was centrifuged at 27,000 \(\times\) g for 15 min at 4°C, after which the supernatant solution was discarded. The membrane pellets were washed twice by resuspending them in the same volume of 5 mM phosphate used initially and centrifuging again. Lipids were obtained from these membrane preparations by chloroform-methanol extraction (2) and quantitated.

† Contribution no. 1629 to the Army Research Program on Antiparasitic Drugs.
drug concentrations by previously described methods (6, 8). The recovery of drug in these experiments exceeded 90% of the amounts added at the beginning of dialysis.

A nonspecific protease from Streptomyces griseus (type VI), phospholipase C from Bacillus cereus (type V), and purified phospholipids were obtained from Sigma Chemical Co. The l-α-phosphatidylcholine was from egg yolk (type IX-E), the l-α-phosphatidylserine was from soybeans (grade I), and the l-α-phosphatidyl-l-serine was from bovine brain.

RESULTS

In equilibrium dialysis experiments (not shown), no high-affinity binding of mefloquine to hemoglobin was detected. By contrast, mefloquine binding to isolated erythrocyte membranes was easily detected (Fig. 1). The concentrations of mefloquine used in these experiments were too low to saturate the membrane binding sites, but subsequent experiments (Fig. 2 and 3) demonstrated that the binding was saturable with an apparent $K_d$ of $3 \times 10^{-7}$ M. Detailed studies of mefloquine binding to intact erythrocytes have been described previously (8).

When erythrocytes were stripped of most of their protein, except band 3, mefloquine binding was undiminished (Fig. 2). Moreover, further treatment of the stripped membranes with a nonspecific protease left no detectable band 3 or peptide fragments as assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, yet there was almost no effect on mefloquine binding (lower panel of Fig. 2), indicating that the binding was not dependent on the presence of protein. By contrast, treatment of stripped membranes with phospholipase C abolished high-affinity binding of mefloquine (upper panel of Fig. 2).

In agreement with the effect of phospholipase C, mefloquine binding to lipids extracted from normal erythrocyte membranes was easily demonstrated (Fig. 3). For comparison, studies of lipids from erythrocytes infected either with chloroquine-resistant or with chloroquine-resistant P. berghei also are shown in Fig. 3. In each case, mefloquine binding to lipid was sufficient to account for most, if not all, of the mefloquine bound to erythrocyte membranes (Fig. 3). The capacity to bind mefloquine was approximately 80 nmol per mg of lipid for each of the preparations. The total amounts of lipid recovered from the preparations were different, however. In two separate experiments with each of the preparations, the total lipid concentrations were: 4.1 and 4.6 mg/g (wet weight) for packed normal erythrocytes; 7.4 and 7.6 mg/g for preparations containing 600 or 1,200 chloroquine-susceptible parasites per 1,000 erythrocytes; and 6.4 and 7.1 mg/g for preparations containing 500 or 600 chloroquine-resistant para-
sites per 1,000 erythrocytes. These results indicate that the previously reported increase in capacity of parasitized erythrocytes to bind mefloquine (8) is due to an increase in lipid content.

Because phospholipids account for much of the total lipid of biological membranes, purified phospholipids were studied next. The capacities of phosphatidylinositol and phosphatidylserine to bind mefloquine with high affinity were 300 to 400 nmol/mg and were much greater than the capacity of phosphatidylcholine (~100 nmol/mg), but all three phospholipids had similar affinities for the drug (Fig. 4). The apparent $K_d$ was $3 \times 10^{-7}$ M. In other experiments (not shown), mefloquine binding to phosphatidylethanolamine was similar to the binding to phosphatidylcholine, but little or no high-affinity binding of mefloquine to phosphatidic acid or to sphingomyelin was detected.

Figure 5 shows studies of chloroquine binding to purified phospholipids. The amount of chloroquine bound per milligram of lipid was small in comparison to mefloquine when the concentration of chloroquine was in the micromolar range (lower panel). From millimolar concentrations,
large amounts of chloroquine were bound (upper panel). In addition to binding to phospholipids, mefloquine bound with high affinity to the chloroquine receptor FP (Fig. 6). In fact, the apparent $K_d$ for binding to FP was indistinguishable from the $K_d$ for binding to erythrocyte membrane lipids ($\sim 3 \times 10^{-7}$ M). For comparison, chloroquine bound to pure FP with a $K_d$ of $3.5 \times 10^{-9}$ M (3).

**DISCUSSION**

Cationic, amphiphilic drugs such as chloroquine are known to concentrate in lysosomes (1, 5) and to cause the accumulation of phospholipids in these organelles (14, 23). In particular, there is an increase in acidic phospholipids, including phosphatidylinositol and bis(monoacylglycerol) phosphate (17, 24). Associated with drug and phospholipid accumulation is inhibition of lysosomal phospholipases A and C (16), possibly because the drugs bind to phospholipids and make them inaccessible as substrates. The extensive high-affinity binding of mefloquine to phospholipids described here is of interest, therefore, not only because it may lead to an understanding of the mode of action of the drug but also because it may provide a useful model for study of the interaction of amphiphilic drugs with membrane phospholipids. Ionic attractions and lipid solubility apparently are involved (12, 15). Otherwise, the interactions are incompletely understood, and they may be different for intact biological membranes than for phospholipids dispersed in the form of liposomes (4).

In addition to providing a promising model for study of drug-phospholipid interactions, knowing that mefloquine binds with high affinity to phospholipids as well as to FP helps explain why this drug behaves differently from chloroquine in biological systems. Clearly, the large accumulation of mefloquine and the lack of a difference between chloroquine-resistant and chloroquine-resistant *P. berghei* with respect to mefloquine accumulation are attributable to binding to phospholipids. Furthermore, the lack of an energy requirement to bind large amounts of mefloquine to phospholipids may obscure an energy requirement for the binding of relatively small amounts of mefloquine to FP. Thus, an energy requirement to make FP accessible to mefloquine in malaria parasites could go undetected.

**FIG. 4.** Binding of mefloquine to phospholipids. Purified phospholipids were dissolved in chloroform or chloroform-methanol (2:1, vol/vol), dried in a glass tube, and dispersed in standard medium, pH 7.4, by vigorous agitation in a Vortex mixer (2 mg of lipid per ml). Mefloquine binding was measured by equilibrium dialysis. Symbols: (○) Phosphatidylserine; (◇) phosphatidylinositol; (□) phosphatidylcholine.

**FIG. 5.** Binding of chloroquine to phospholipid. Purified phospholipids were dispersed in standard medium, pH 7.4, as described in the legend to Fig. 4, and chloroquine binding was measured by equilibrium dialysis. Symbols: (○) phosphatidylcholine; (◇) phosphatidylinositol. The two panels show different concentration ranges of free chloroquine (external chloroquine).
Whether energy is required or not, mefloquine and chloroquine binding to FP also helps explain the behavior of these drugs in biological systems. For example, the $K_c$ of $10^{-6}$ M for mefloquine, which was estimated from the inhibition of chloroquine binding to erythrocytes infected with chloroquine-susceptible *P. berghei* (7), is reasonable since the $K_c$ for mefloquine binding both to phospholipid and to FP is approximately the same. Likewise, it is reasonable for the apparent $K_c$ for chloroquine binding to erythrocytes infected with chloroquine-susceptible *P. berghei* (6) to be much smaller than the apparent $K_c$ for chloroquine as an inhibitor of mefloquine binding (8) because chloroquine binds much more tightly to FP than to phospholipids.

With regard to the mode of action of mefloquine as an antimalarial drug, high-affinity binding both to phospholipids and to FP raises the possibility that the drug acts in two different ways. It may share the same mode of action as chloroquine for those parasites that produce FP, and it may have a second mode of action based upon its interaction with phospholipids. Consistent with a second mode of action, malaria parasites are enriched in phosphatidylinositol (13, 20), which could make them especially vulnerable to mefloquine. Further consideration of the possibility of a second mode of action is warranted, for it could explain the superiority of mefloquine in the treatment of chloroquine-resistant malaria.

**ACKNOWLEDGMENT**

This work was supported by a contract from the U.S. Army Medical Research and Development Command (DADA 17-72-C-2008).

---

**LITERATURE CITED**

