Chloroquine-Resistant *Plasmodium falciparum*: Difference in the Handling of $^{14}$C-Amodiaquin and $^{14}$C-Chloroquine

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$^{14}$C-amodiaquin and $^{14}$C-chloroquine were used to study drug binding by preparations of owl monkey erythrocytes infected either with a chloroquine-susceptible (CS) or with a chloroquine-resistant (CR) strain of *P. falciparum*. Both of these drugs are derivatives of 4-aminoquinoline, but they differ in their side chains, and there are differences in the way they are handled by preparations of erythrocytes infected with malaria parasites. Although the apparent association constant for the binding of either drug is approximately $10^7$ mol$^{-1}$, two to three times more radioactivity was bound from $^{14}$C-amodiaquin than from $^{14}$C-chloroquine. Furthermore, there was no apparent difference between CS and CR parasites with respect to $^{14}$C-amodiaquin binding, whereas erythrocytes infected with CR parasites have a deficiency of $^{14}$C-chloroquine binding. This difference in the handling of amodiaquin probably accounts for its superiority in the treatment of the owl monkey and of humans infected with CR *P. falciparum*.

More than 2 years ago, Geiman and associates (Q. M. Geiman, personal communication) demonstrated that amodiaquin inhibits the growth in vitro of the highly chloroquine-resistant (CR) Vietnam Monterey (Fort Ord) strain of *Plasmodium falciparum*. This observation was confirmed by Rieckmann (4), who used a different experimental technique and the CR Marks strain of *P. falciparum*. In a follow-up to Geiman’s work, Schmidt and associates in a presentation before the American Society of Tropical Medicine and Hygiene at San Francisco in 1970 described studies of the therapeutic effectiveness of amodiaquin in owl monkeys (*Aotus trivirgatus*) infected with CR strains of *P. falciparum*. Using doses of amodiaquin tolerated well by owl monkeys, they were able to cure experimental infections with the Vietnam Monterey, the Vietnam Oak Knoll, and the Malayan IV strains. By contrast, none of these strains is susceptible to the maximum doses of chloroquine tolerated by owl monkeys. Amodiaquin also is superior to chloroquine in the treatment of human infections with *P. falciparum*, as has been demonstrated by Rieckmann (4) in studies of volunteers infected with the Marks strain. Although superior to chloroquine, amodiaquin is not as effective in treating infections with CR strain of *P. falciparum* as it is in treating infections with chloroquine-susceptible (CS) strains (4; L. H. Schmidt et al., submitted for publication).

The superiority of amodiaquin in treating chloroquine-resistant malaria is of interest because amodiaquin and chloroquine differ structurally only in their side chains. Both drugs are derivatives of 7-chloro-4-aminoquinoline and both have side chains terminating in a diethylamino group (Fig. 1). In amodiaquin, the diethylamino group is attached to 4-aminoquinoline through an o-cresol, as illustrated in Fig. 1; in chloroquine, the attachment is through an isopentyl group. This structural difference evidently affects the interactions of these drugs with malaria parasites.

An important interaction of chloroquine with CS malaria parasites is its binding to a drug receptor (2, 3), which probably is located on or near food vacuoles (1). Recent studies of this interaction demonstrated that owl monkey erythrocytes infected with CS strain of *P. falciparum* could bind 3.8 μmol of chloroquine per kg of cells (wet weight) with an apparent association constant of approximately $10^7$ mol$^{-1}$ (3). In comparison, erythrocytes infected with a CR strain of *P. falciparum* could
bind only 1.1 \mu mol of chloroquine with similar affinity. These and other observations (Fitch, Proc. Helminthol. Soc. Wash., Suppl., in press) provide support for the hypothesis that binding of chloroquine and related drugs to a high-affinity drug receptor is essential for their chemotherapeutic action. To investigate the relationship between drug binding and therapeutic effectiveness, the following studies of \(^{14}\)C-amodiaquin binding were undertaken.

**MATERIALS AND METHODS**

Owl monkeys in routine passage lines either for the CS Malayan Camp-CH/Q strain or for the CR Vietnam Oak Knoll strain of *P. falciparum* were provided by L. H. Schmidt; uninfected monkeys served as an additional control. The monkeys were infected by intravenous administration of blood containing approximately 10\(^4\) parasites, and blood was obtained for study 9 to 12 days later when the parasitemias ranged from 1,320 to 2,880 parasites per 10,000 erythrocytes.

Radiochemically pure \(^{14}\)C-amodiaquin [4-((7-chloro-4-quinolyl) amino)-\(\alpha\)-(diethylamino)-\(\alpha\)- cresol-\(\alpha\)-\(^{14}\)C] with a specific activity of 1.21 mCi/mmoll was provided by A. J. Glazko. The molecule was labeled in the methylene bridge as shown in Fig. 1.

![Structural formulas of amodiaquin and chloroquine. Asterisks mark the location of \(^{14}\)C in the labeled drugs.](attachment:b.png)

The methods used to measure amodiaquin binding by preparations of washed erythrocytes in vitro were similar to those already described for studies of chloroquine binding (2, 3). Thus, washed erythrocytes were incubated for 30 to 60 min in media containing graded amounts of \(^{14}\)C-amodiaquin. At the end of incubation, the cells were separated from medium by centrifugation, and a sample of the medium was transferred to a counting vial for measurement of radioactivity with a liquid scintillation spectrometer and a xylene-dioxane-2-ethoxyethanol (1:3:3) counting solution. The channels ratio method was used to correct for quenching by these aqueous solutions. All samples were counted for a sufficient length of time to achieve a probable error of counting of 2% or less.

The amounts of radioactivity in erythrocyte pellets were calculated by subtracting the amount of radioactivity in the medium at the end of incubation from the amount added at the beginning of incubation. In latex experiments in which mouse erythrocytes infected with *P. berghei* were used, 95% or more of the radioactivity calculated in this way to be present in erythrocyte pellets was recovered when nonradioactive chloroquine was added to the pellet at the end of incubation to displace the radioactive amodiaquin or its metabolites.

In the present studies, some of the preparations of erythrocytes were divided into a sufficient number of parts to allow chloroquine and amodiaquin binding to be measured concurrently. The methods used to measure chloroquine binding have been described in detail previously (2). The chloroquine-\(^{3}\) \(^{14}\)C used in these studies had a specific activity of 1.71 mCi/mmoll and was obtained from New England Nuclear Corp. The position of the label in the chloroquine molecule is shown in Fig. 1.

**RESULTS**

The accumulation of radioactivity in erythrocytes incubated with \(^{14}\)C-amodiaquin is shown in Fig. 2. Only values from 60-min periods of incubation are shown, as there was no difference between 30- and 60-min values, i.e., steady-state conditions prevailed. Since metabolism of amodiaquin may have occurred during incubation, it is possible that the radioactivity in pellets and medium does not represent amodiaquin alone. For this reason, in Fig. 2 the compounds measured are not named. Nevertheless, it is clear that erythrocytes infected with malaria parasites accumulated much more radioactivity than uninfected erythrocytes. Furthermore, no difference between the strains of parasites is evident, although the range of values from monkeys infected with CS parasites is large. A large range of values also was observed previously in studies of chloroquine binding (3).

From the data shown in Fig. 2, the maximal capacity of preparations of erythrocytes infected with *P. falciparum* to bind amodiaquin or its metabolites with an apparent association constant of approximately 10\(^7\) is estimated to be 10 \mu mol per kg (wet weight). This value is two to three times greater than the maximal capacity of comparable preparations of erythrocytes infected with CS *P. falciparum* to bind chloroquine with similar affinity (cf. Fig. 2 and 3).

The results of studies of chloroquine binding are shown in Fig. 3. These additional studies were needed because the CR strain of *P. fal*
ciparum used in the present work was different from the Vietnam Monterey strain used in the earlier study (3). To facilitate comparison, the new data for chloroquine binding are superimposed on a previously published figure that summarizes the older data. This comparison demonstrates that the Oak Knoll strain of *P. falciparum* has a deficiency of chloroquine binding similar to that of the Monterey strain. It is noteworthy that erythrocyte preparations which bound the least radioactivity from ¹⁴C-amodiaquin also bound the least ¹⁴C-chloroquine. Whether or not the ¹⁴C-chloroquine was metabolized was not determined in these studies.

**DISCUSSION**

As would be predicted from structural similarities (Fig. 1), amodiaquin competes with chloroquine for the high-affinity drug receptor of *P. berghei* (Fitch, Proc. Helminthol. Soc. Wash., Suppl., in press), and, as would be predicted for two drugs acting through the same receptor, CR *P. berghei* exhibits cross-resistance to amodiaquin (5). Because CR *P. falciparum* also exhibits a degree of cross-resistance to amodiaquin, it is tempting to conclude by analogy that amodiaquin and chloroquine are served by the same drug receptor in *P. falciparum*. This conclusion is not justified, however, until the differences in handling of ¹⁴C-amodiaquin and ¹⁴C-chloroquine (Fig. 2 and 3) are adequately explained. These differences could be explained either by greater access of amodiaquin to a single type of receptor serving both drugs or by the existence of two types of receptors, one for each drug. In the former case, intact ability to accumulate radioactivity from ¹⁴C-amodiaquin (Fig. 2) despite a deficiency of chloroquine binding (Fig. 3) could be interpreted as evidence that the receptor is present but inaccessible to chloroquine in CR parasites.

Regardless of whether there are two types of receptors or only one, the intact ability of erythrocytes infected with CR *P. falciparum* to accumulate radioactivity from ¹⁴C-amodiaquin probably is related to amodiaquin’s therapeutic superiority. This finding also would be compatible with one or more of the following four possible types of adaptations: (i) a change in the metabolism of amodiaquin, (ii) decreased susceptibility of vulnerable metabolic pathways to amodiaquin, (iii) use of preexisting, less vulnerable, alternate metabolic pathways, and (iv) a modification of the drug receptor which would render the complex of drug with receptor less harmful. Any one of these adaptations could account for the relative resistance of CR *P. falciparum* to amodiaquin. If amodiaquin and chloroquine are served by the same receptor, the

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**Fig. 2. Amodiaquin accumulation by erythrocytes.** Five percent suspensions of washed erythrocytes were incubated for 1 h under room air at 22 C and pH 7.4 in an aqueous medium of the following composition (millimoles per liter): NaCl, 25; KCl, 4.8; MgSO₄, 1.2; glucose, 86; and Na₂HPO₄·50; the pH was adjusted with HCl, and ¹⁴C-amodiaquin was added to achieve the desired concentrations of the drug. Left panel: three uninfected monkeys. Center panel: monkeys infected with the Vietnam Oak Knoll strain of *P. falciparum*. Right panel: monkeys infected with the Malayan Camp-CH/Q strain of *P. falciparum*. The different symbols for infected monkeys represent different monkeys. All of the data for infected monkeys are corrected to represent 5,000 parasites per 10,000 erythrocytes. The amount of radioactivity, expressed as micromoles (calculated on the basis of the specific activity of the ¹⁴C-amodiaquin) per kilogram (wet weight) of erythrocyte pellet, is shown.
deficiency of chloroquine binding observed in these and in earlier experiments (3) would favor modification of that drug receptor as the cause of resistance of certain strains of P. falciparum to derivatives of 4-aminoquinoline.

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LITERATURE CITED