INTRODUCTION

For decades, the 4-aminoquinoline chloroquine (CQ) was the mainstay for the prevention and treatment of malaria because of its low cost, safety, and efficacy. However, CQ-resistant Plasmodium falciparum has now been reported from almost every malaria endemic country, and this drug can no longer be considered appropriate for the treatment of malaria in many areas (1). Reports of CQ-resistant P. vivax also have begun to emerge from Asia and South America (2). In response to the problem of CQ resistance, the quinolinemethanol mefloquine has been widely deployed and used for the prevention and treatment of malaria in areas where CQ is no longer effective. However, resistance to this drug has emerged in many malarious regions (3–8). Although the older quinolinemethanols quinine and quinidine remain useful in areas of CQ and mefloquine resistance, these drugs have been losing efficacy, and cases of cross-resistance among the different quinolines have been described (9–16).

The impact of resistance to CQ and other quinoline antimalarials is measured not only by the reduction in their efficacy but also by the increasing necessity to rely on the more expensive and more toxic current alternatives. Strains of P. falciparum from Cambodia and Thailand exist that are resistant to all available antimalarials except the artemisinin compounds (1). Given the great success of the quinolines prior to the emergence of resistance, strategies to develop new antimalarials should include new agents that act upon the target of these drugs as well as chemosensitizers that can reverse the resistance phenotype. A better understanding of underlying mechanisms of quinoline resistance should assist these strategies. This chapter reviews current understanding and controversies regarding the mechanisms of resistance to the quinoline antimalarials, with a focus on P. falciparum and the most widely used drugs of this class.

MECHANISM(S) OF CHLOROQUINE RESISTANCE

Introduction

Despite massive drug pressure following its introduction in the 1940s, the resistance of P. falciparum to CQ was not recognized until the late 1950s, when treatment failures were reported from distinct foci in Southeast Asia and South America (17). Resistance
has steadily spread from these two foci and now is established in almost all malarious areas around the world (1). The emergence of CQ resistance only after many years of widespread CQ use suggests that multiple mutations are required to produce the CQ resistance phenotype (18). It is now generally agreed that CQ acts by disrupting the detoxification of heme in the parasite food vacuole (see Chapter 6). The mechanism of resistance to CQ is more controversial. From a biochemical standpoint, CQ-resistant parasites have been found to accumulate less CQ compared to CQ-sensitive parasites (19–23). This difference can be modified by a number of chemosensitizing agents such as the Ca\textsuperscript{2+} channel blocker verapamil (24). Recent work has attempted to investigate the problem from a genetic standpoint, with the goal of identifying genes that encode proteins that play a role in CQ resistance (25–27).

**Chloroquine Resistance and Drug Accumulation**

It has been suggested that the accumulation of CQ in the *P. falciparum* food vacuole, which underlies the action of the drug, is related to the binding of CQ to free heme (Chapter 6). Once in the food vacuole, CQ appears to form complexes with heme in its hematin (\(\mu\)-oxodimer) form and interrupt the normal polymerization of heme as it is released upon hemoglobin digestion. These CQ-hematin complexes are thought to engender parasite toxicity (Chapter 6).

Mechanisms of CQ resistance among different *Plasmodium* species may vary. In murine parasites, such as *P. berghei*, CQ resistance is associated with a decrease in the formation of hemozoin (pigment), which consists of polymerized (and thus detoxified) heme particles (28). However, there are no clear differences in the quantity of hemozoin in CQ-resistant and CQ-sensitive *P. falciparum* (29).

A number of hypotheses have been proposed to explain differences in CQ accumulation between resistant and sensitive parasites. One theory is that decreased accumulation is the result of increased CQ efflux from CQ-resistant parasites, which actively remove the drug from its site of action (22,30). This theory was supported by data suggesting that CQ-resistant parasites released preaccumulated CQ almost 50 times faster than CQ-sensitive isolates (30,31). These results were considered consistent with the finding that verapamil partially reversed resistance and reduced the apparent rate of drug efflux from CQ-resistant parasites (30,32). CQ resistance was therefore proposed to involve a mechanism analogous to that mediated by P-glycoprotein molecules in mammalian tumor cells (discussed in more detail below) (32). More recent reports, however, found that CQ efflux rates in CQ-resistant and CQ-sensitive strains are similar and that the decreased steady-state levels of CQ in resistant strains are the result of a diminished level of accumulation rather than a drug export mechanism (23,33–35).

Several hypotheses incorporate the concept that decreased CQ accumulation can be the result of diminished drug accumulation. The uptake of diprotic CQ in acid food vacuoles because of a weak-base ion-trapping mechanism is predicted by the Henderson–Hasselbach equation (21,36,37). Increases in vacuolar pH could thus explain decreased accumulation of CQ in resistant parasites (34,38). An increase in vacuolar pH of 0.5 pH units was proposed to be sufficient to decrease CQ accumulation by 10-fold (35). Reports of increased vacuolar pH have been explained by a weakened proton pump, which was thought to be consistent with results showing CQ-resistant parasites to be more susceptible than CQ-sensitive parasites to the proton-pump inhibi-