

# 9

## *Modes of Action of Antiprotozoal Agents*

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### 1. INTRODUCTION

There are relatively few antiprotozoal agents compared with the many antibiotics available for the treatment of bacterial infections. Similarities in host and eukaryotic parasite metabolism have made development of specific chemotherapeutic agents difficult and have necessitated the use of agents that often exhibit limited specificity for the parasite and excessive toxicity to the host. This chapter examines the mechanisms by which antiprotozoal agents exert their effects. Information on modes of action of several antiprotozoals is lacking either because little research has been undertaken to define the mechanisms or because primary and secondary drug effects have been difficult to differentiate.

Although currently used antiprotozoal agents often have low specificity and high toxicity, the future holds promise for the development of superior drugs. Ongoing research in the areas of protozoan biochemistry and physiology seeks to define differences between host and parasite metabolism that may be exploited. Such research offers a promise for the development of antiprotozoal agents that are exquisitely targeted. At the end of this chapter metabolic differences are discussed that may be future targets for chemotherapy.

### 2. MODES OF ACTION OF ANTIPROTOZOAL AGENTS

#### 2.1. *Malaria*

Drugs currently used for the treatment of malaria include 4- and 8-aminoquinolines, quinine, and dihydrofolate reductase inhibitors, the latter often in com-

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193

bination with *p*-aminobenzoic acid (PABA) analogues. Two modes of action have been proposed for the aminoquinolines. Chloroquine (CQ) binds to ferriprotoporphyrin IX (FP), a malarial degradation product of hemoglobin present in the food vacuole of the parasite (Chou *et al.*, 1980). FP alone is lytic for malaria, but complexation with CQ increases its lytic properties (Fitch *et al.*, 1982; Dutta and Fitch, 1983). Malarial parasites apparently must sequester FP to prevent autolysis. CQ treatment results in the formation of an FP-CQ complex that may divert FP from its site of sequestration and cause lysis of the parasite (Fitch *et al.*, 1982). This mechanism explains CQ resistance in *Plasmodium berghei*, which does not synthesize FP. However, CQ-resistant *P. falciparum* contains FP, suggesting that FP must be sequestered to the extent that it is unable to bind CQ if this mechanism is correct. An alternative mechanism proposes that aminoquinolines exert their effects by alkalinization of the malarial food vacuole. Malarial parasites generate a pH gradient between the cytoplasm and food vacuole by use of a  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase proton pump common to eukaryotic cells (Warhurst and Thomas, 1978). CQ at physiological pH is nonprotonated and membrane permeable. Diffusion into the acidic food vacuole protonates CQ, which becomes membrane impermeable. Accumulation of the protonated drug alkalinizes the food vacuole and reduces the activity of lysosomal enzymes that require an acidic pH. Inhibition of lysosomal proteases decreases hemoglobin digestion and starves the parasite for amino acids (Home-wood *et al.*, 1972). Excessive food vacuole alkalinization may also increase the permeability of the vacuole membrane and release lysosomal hydrolyases into the cytoplasm. Electron microscopic studies of CQ-treated *P. falciparum* revealed swelling of primary lysosomes and endocytic vesicles within the food vacuole that contain undigested hemoglobin, suggesting that lysosomal enzyme activity is inhibited (Yayon and Ginsberg, 1983). If this mechanism of CQ toxicity is correct, CQ resistance could be related to reduced acidity of the food vacuole and decreased CQ incorporation. Reduced vacuole acidity would explain the lack of visible FP within the vacuole, since pigment clumping is pH dependent (Warhurst and Thomas, 1978). Alternatively, the lipid content of the food vacuole may be modified in CQ-resistant malaria such that CQ diffusion into the vacuole is reduced to a nontoxic level. Further research should determine which of the proposed mechanisms accounts for the antimalarial action of aminoquinolines. Quinine has been used for centuries in the treatment of malaria. Its mode of action has not been well studied, but it is presumed to be the same as that of the aminoquinolines.

Dihydrofolate (DHF) reductase inhibitors are also used in the treatment of malaria, often in combination with PABA analogues (sulfonamides). The mode of action of these folate biosynthesis inhibitors was reviewed by Hitchings and Burchall (1965) and is presented in Fig. 1. Sulfonamides inhibit the synthesis of dihydropteroic acid from pteridines, PABA, and glutamate; 2,4-diaminopyrimidines inhibit the reduction of dihydrofolate to tetrahydrofolate (THF) by dihydrofolate reductase. Inhibition of THF synthesis prevents conversion of uracil to thymine. Thymine is required for DNA synthesis.

The modes of action of the diaminopyrimidines pyrimethamine and trimethoprim have been examined in the plasmodia. Feron *et al.* (1969) partially purified the DHF