Chapter 39
Drug Resistance Mechanisms in *Entamoeba histolytica, Giardia lamblia, Trichomonas vaginalis,* and Opportunistic Anaerobic Protozoa

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1 Introduction

Resistance of organisms to toxic agents is a survival mechanism fundamental for adaptation and evolution of life. As a counterpart, drug resistance is a medical problem in cancer and infectious diseases, with not many alternatives available. *Entamoeba histolytica*, *Giardia lamblia* (syn. *duodenalis* or *intestinalis*), and *Trichomonas vaginalis* (Fig. 1) are anaerobic and microaerophilic pathogens capable of developing drug resistance. Over one billion individuals worldwide harbor these and other anaerobic protozoa such as *Blastocystis hominis*, *Cryptosporidium parvum*, *Isospora* spp., *Cyclospora* spp., and *Microsporidia* (1). Most infected people live in poor countries. Unhygienic sanitary conditions and poor health education are the causes for infectious protozoan prevalence, and they can be eradicated by implementing drainage, parasite-free water supply, and sexual education for all people.

E. histolytica and *G. lamblia* (Fig. 1a, b) enter humans by ingestion of cysts that come out with feces from infected individuals. Host factors induce transformation of cysts into trophozoites which cause the diseases. A high percentage of infected people do not present symptoms, but spread the parasite. The cysts, highly resistant to atmospheric conditions, are formed in the intestine and excreted with feces. They contaminate water and food, their vehicles to infect other hosts. *E. histolytica* mainly invades gut and liver, but also the brain, lungs, skin, and genitals. *T. vaginalis* (Fig. 1c) is the causative of the most common nonviral human sexually transmitted disease (2). Between 25% and 50% of infected people are asymptomatic, but the infection provokes vaginitis with inflammatory discharge and predisposition to cervical neoplasia, and it causes complications during pregnancy and results in low weight of newborns, preterm delivery, and respiratory diseases. In men, it produces urethritis, orchitis, oligoathenosteratospermia, and hypogonadism (3). Trichomonosis is linked to an increased risk of cytomegalovirus (CMV) (4) and human immunodeficiency virus (HIV) transmission (5).

Anaerobic protozoa emerged very early in evolution, and parasites have gained many characteristics through coevolution inside the host. They share some features, but also present striking differences. *E. histolytica* has a cytoplasm full of vacuoles, and except for the nucleus, organelles are difficult to distinguish in the highly phagocytic trophozoites (Fig. 1a). *Giardia* has eight flagella, two nuclei, and a ventral disk formed by giardins and other cytoskeleton proteins that allow parasite adherence to epithelia (Fig. 1b). *T. vaginalis* has four anterior flagella and a recurrent flagellum incorporated into an undulating membrane (Fig. 1c). It can form pseudopodia to phagocyte epithelial cells. The three parasites use adherence molecules and cysteine proteases to colonize and damage tissues (6–8). They do not have mitochondria and peroxisomes, organelles found in most eukaryotes, or canonical mitochondrial processes. *E. histolytica* has a double membrane-limited organelle called EhkO, which contains DNA and pyruvate: ferredoxin oxidoreductase (PFOR) (9). Additionally, mitochondria-like enzymes have been found in others organelles called mitosome and crypton (10, 11). Similarly, it has been reported that *G. lamblia* contains mitosomes that function in iron–sulfur protein maturation (12). *T. vaginalis* has the hydrogenosome, where both decarboxylation of pyruvate by PFOR and energy generation take place (13). Phylogenetic analysis suggest that *E. histolytica* and *G. lamblia* iron-hydrogenase genes were derived from a common eubacterial ancestor, distinct from the *T. vaginalis* iron-hydrogenases genes ancestor (14). Similarity in their metabolism allows the use of common drugs, such as the 5-nitroimidazoles, to kill them.

Metronidazole (1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole), the preferred drug for the three mentioned parasites, enters the cell by passive diffusion in an inactive form. It has a lower redox potential (–460 mV) than ferredoxin (Fd) (–320 mV), gains electrons transferred by PFOR to Fd to be

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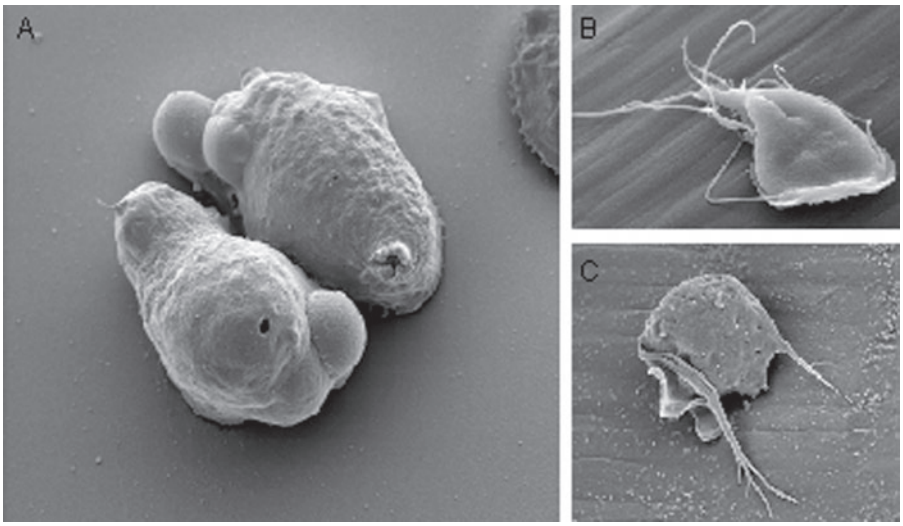

Fig. 1 Scanning electron microscopy images of (**a**) *E. histolytica*, (**b**) *G. lamblia*, and (**c**) *T. vaginalis* trophozoites. (Micrographs were kindly provided by Dr. Arturo González Robles, Departamento de Patología Experimental, CINVESTAV IPN, México)

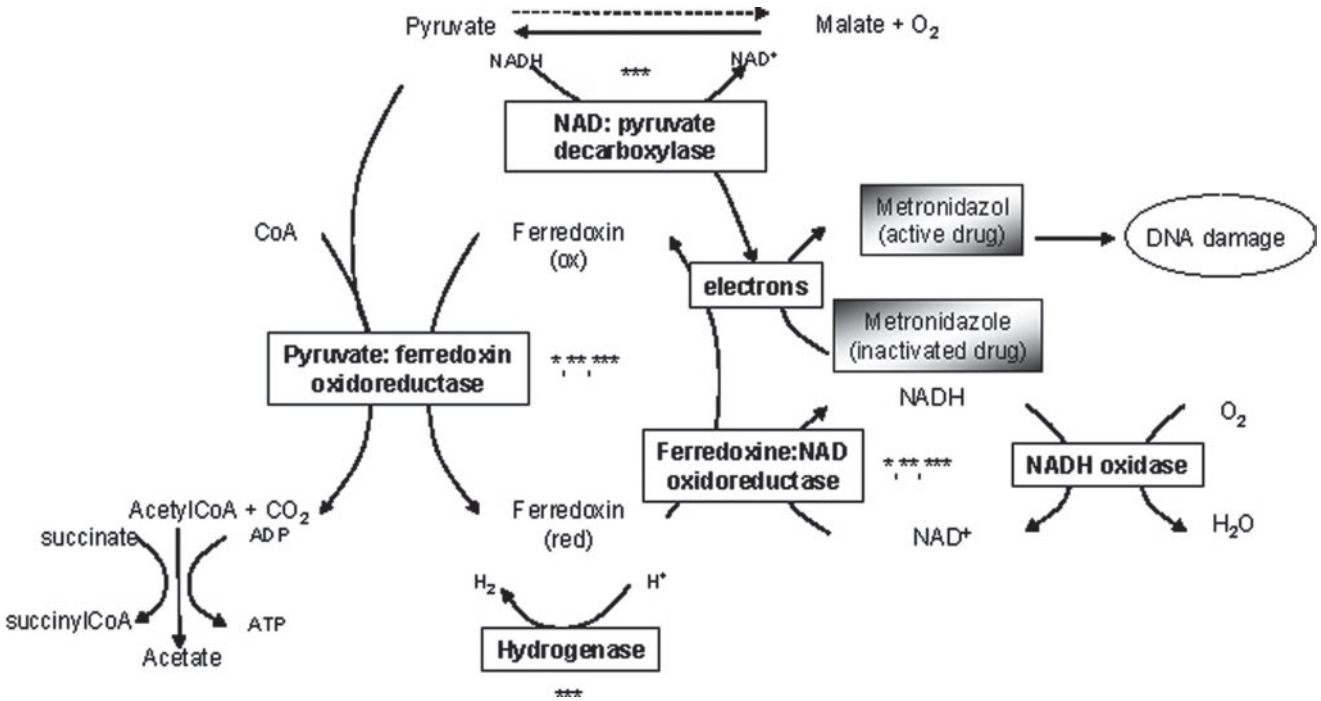

Fig. 2 Terminal part of the glycolytic pathway of anaerobic protozoa and its relation with metronidazole activation. *Represents the current knowledge on enzymes involved in drug activation. **E. histolytica*, ***G. lamblia*, ****T. vaginalis*. (Adapted from Refs. 1 and 66)

converted to toxic nitro or nitroso anions or intermediates, such as hydroxylamines (Fig. 2), and creates a concentration gradient favoring drug accumulation in the cell (15, 16). Reduced metronidazole binds DNA and interferes with respiration and motility (15–17). However, because of its toxicity and the emergence of metronidazole-resistant protozoa (18–21), new efficient drugs are needed.

Protozoa use various drug-resistance mechanisms, including DNA mutations, modulation of enzymes, and pump-like protein expression, such as the P-glycoproteins (PGPs) involved in the multidrug resistance (MDR) phenotype, described in many organisms (22). As poor countries cannot implement public health measures to prevent the spread of parasites, it is very important to understand parasite drug resistance mechanisms and find the way to bypass them, as well as to generate new drugs and vaccines. Here we review the current knowledge on drug susceptibility in the anaerobic protozoa causative of human diseases.