Chemotherapeutics of Neglected Waterborne Parasites: Current Status and Future Perspectives

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Abstract

Waterborne protozoan parasites Cryptosporidium and Giardia cause diarrheal diseases worldwide. Cryptosporidiosis and giardiasis cause significant disease burden and economic impact much of the developed world and their treatment options are limited. These diseases are, therefore, included in the World Health Organization neglected disease initiative. These protozoans are also included as bioterrorism agents because of their potential ability for large-scale contamination of water supplies. This mini review discusses ways for therapeutic options to control the diseases caused by these parasites.

Keywords: Protozoan parasites; Chemotherapy; Neglected diseases; Bioterrorism; Cryptosporidium; Giardia

Enteric diseases caused by waterborne protozoan parasites afflict millions of people in much of the developing world. Even in industrialized nations, enteric protozoan parasites, contribute to significant public health problems. Protozoan species, Cryptosporidium and Giardia, are responsible for a considerable worldwide morbidity and mortality due to diarrheal diseases leading to malnutrition. Because contaminated water supplies can potentially be used for bioterrorism, these parasites, Cryptosporidium and Giardia are listed as a category B bioterrorism agent. Drugs available to control these diseases, particularly cryptosporidiosis, are limited. Therefore, these diseases are listed in the World Health Organization’s list of neglected diseases (1) and there is a need to develop a portfolio of new generation of therapeutics for better management.

Cryptosporidium

Life Cycle

Cryptosporidium is a protozoan parasite that belongs to the subphylum Apicomplexa whose better-known member is the malaria parasite (2). The human infection is caused by two species, C. hominis that infects only humans, and C. parvum that also infects other mammals (3). Unlike other apicomplexans, Cryptosporidium species can complete their asexual and sexual lifecycle stages in a single host. The lifecycle is initiated upon ingestion of oocysts. The acidic environment in the stomach activates oocysts to release proteases and peptidases and excyst. Excystation causes the release of sporozoites, the infective form, in the stomach and upper intestine. Proteolytic enzymes are likely to play a major role in host cell invasion as well as for egress similar to that seen in apicomplexans Plasmodium and Toxoplasma (3-5).

Recently, it has been shown that a subtilisin-like serine protease, CpSUB1, localizes to the apical region in sporozoites and may have a crucial role in the host cell invasion by C. parvum (6). The sporozoites are motile and adhere to intestinal epithelial cell receptors. The parasite induces polymerization of the host cell actin and this event is an essential step for the internalization of the sporozoite (7, 8). The parasite resides inside a parasitophorous vacuole within the epithelial cells of the microvilli to matures into trophozoites, undergo multiple division during schizogony to form type I merozonts. Merozoites rupture from meronts to reinvade other epithelial cells. During the sexual cycle merozoites differentiates into micro and macrogamonts. Fertilization of macrogamonts by the microgamonts results in the formation of the infective oocysts.
Pathogenesis and Epidemiology

The watery diarrhea that results following Cryptosporidium infection is caused by defects in Na+ and H2O absorption and increased Cl- secretion (9). The parasite induces NFκB expression to facilitate its survival in infected cells (10). Infection results in proinflammatory cytokine response and elevated expression of other related proteins involved in inflammatory response, such as tumor necrosis factor α, interleukins and interferone γ leading to dysfunctional intestinal permeability (11, 12). Although the disease can be asymptomatic in immunocompetent individuals, cryptosporidiosis can be severe in immunocompromised people such as those with AIDS, children and during pregnancy.

It is estimated that as many as 300,000 individuals are infected each year although only about 3000 annual cases are usually reported (13, 14). The under reporting is likely due to the fact that many infected individuals are asymptomatic. In 1993 there was a massive outbreak in Milwaukee because of failure of water treatment plants that resulted in over 400,000 people being infected with Cryptosporidium, majority of whom had watery diarrhea (15). Minor outbreaks in Florida have also been reported in 2006 (16, 17).

Current Therapeutic Options

Nitazoxanide, a nitrothiazolyl-salicylamide derivative, has been shown to be active against C. parvum, both in vitro and in vivo (18). The drug showed promise in AIDS-related cryptosporidiosis (19). Nitazoxanide has been shown to reduce the duration of diarrhea and oocyst shedding compared to placebo (20). Nitazoxanide is a prodrug, which upon absorption is metabolized to the active form tizoxanide (desacetyl-nitazoxanide). Nitazoxanide has been shown to be a noncompetitive inhibitor of pyruvate:ferredoxin/flavodoxin oxidoreductases in Cryptosporidium, Entamoeba and Giardia (21). It is likely that the molecular target of the drug in Cryptosporidium will be similar as this anaerobic pathway is available in C. hominis based on the genome sequence information (22).

The aminoglycoside antibiotic paromomycin is the only other drug that demonstrated modest activity against Cryptosporidium (23, 24). Although macrolide antibiotics such as spiramycin, azithromycin, roxithromycin, and clarithromycin were initially thought to be promising (25-27), the effectiveness of these drugs in randomized clinical trials have yet to be proven beyond doubt (20).

Future Perspective

It is evident that other than nitazoxanide and paromomycin, treatment options for cryptosporidiosis are limited; therefore, there is an urgent need to develop new generation of therapeutics. Because validation of new molecular targets is impossible in Cryptosporidium as the parasite cannot be maintained in continuous culture and lack of genetic manipulation tools, the “piggy back” approach can be an alternate route to identify new therapeutics. In this approach the knowledge of chemical entities that were developed other diseases can be used for the development of new drug leads for cryptosporidiosis. Using this philosophy the nitrogen-containing bisphosphonates (N-BPs) that are approved drugs for bone disorders has been tested for anticryptosporidium activities and has been shown to be inhibitory to Cryptosporidium growth in low micromolar concentrations (28). N-BPs are inhibitors of farnesyl pyrophosphate synthase (FPPS) (29). The isoprenoid pathway has also been considered to be the target of N-BPs in other apicomplexan parasites, Plasmodium and Toxoplasma (30, 31). Although there are three prenyl synthases is encoded by the Cryptosporidium genome (28), the pathway for the synthesis of substrates for these enzymes in not clear as both the classical mevalonic acid pathway and the alternative methylerythritol phosphate pathways are absent (32). It has been shown that the target for N-BPs in Cryptosporidium is a novel nonspecific polyprenyl pyrophosphate synthase that can synthesize isoprenoids from C20->C45 (28).

The uniqueness of Cryptosporidium metabolic pathways among apicomplexans has been evident from its genome sequence analysis of the 9Mb genome distributed into 3,950 genes on 8 chromosomes (22, 33). Many conventional drug targets apicoplast-related metabolic pathways such as, shikimate pathway, the mannitol cycle and hypoxanthine-xanthineguanine phosphoribosyltransferase (HXGRT), (22, 33). Cryptosporidium lacks the biosynthetic machinery for the synthesis of purines, pyrimidines and amino acids, therefore, is dependent on scavenging from the host through a variety of transporters. These transporters are likely to be important for the survival of the parasite and as such will be ideal therapeutic targets (22, 33). It has a bacterial-type inosine-5’-monophosphate dehydrogenase (IMPDH) as the only means to convert AMP to GMP. Recently, a high throughput screen targeting divergent NAD site of IMPDH has
resulted in identifying compounds with an IC\textsubscript{50} values as low as 0.13 µM (34). These compounds also showed activity in the infected HCT-8 epithelial cells (34).

Although many classical targets for apicomplexan parasites are absent in Cryptosporidium, the information from its genome sequence has generated optimism that several novel molecular targets exist and those can potentially be exploited to develop new generation of drugs.

**Giardia**

**Life Cycle**

*Giardia* is a flagellated amitochondrial flagellated parasitic protozoa that was first reported Van Leewenhoek in his own fecal sample in 1681 (35). The protozoan was initially named Cercomonas intestinalis but was later named *Giardia lamblia* after A. Giard of Paris and F. Lambl of Prague. The organism is also known as *Giardia intestinalis* or *Giardia duodenalis*. *Giardia* was once thought to be one of the most primitive eukaryote based on eukaryotic tree constructed based on analyses of small subunit ribosomal RNA genes (36), however, a egalitarian view of eukaryotic phylogeny argues that organisms near the base of a tree are not necessarily primitive (37).

*Giardia* has two stages of lifecycle: trophozoite and cyst. The trophozoites, although anaerobe, are aerotolerant. The organism at this stage contains two nuclei at the anteriror end and has an adhesive disk made of spelicular microtubules for adhesion to the brush borders of the intestine. Trophozoites also contain four pairs of flagella that help in locomotion. Trophozoites divide by binary fission in 9-12 hours unless environmental stimuli such as low cholesterol and cholic acid among other unknown agents induce encystation to form four-nucleated cysts (38, 39). Cysts are the infective form and are resistant to most environmental agents and desiccation. Ingested cysts after traversing the acidic environment of gastric mucosa undergo excystation to form motile trophozoites that colonize duodenal or jejunal mucosa.

**Pathogenesis and Epidemiology**

*Giardia* infection results from ingestion of contaminated water or food containing cysts. Person-to-person infection also occurs because of poor fecal-oral hygiene. Many individuals can be asymptomatic, particularly when the parasite burden is low. Symptoms of infection can be quite variable and may include nausea, vomiting, abdominal cramps, flatulence, and diarrhea that usually appears 1-2 weeks after infection, the acute phase of infection lasts 3-4 days but can be often chronic (40). In some patients, *Giardia* infection leads to persistent gastrointestinal problems (41). In children, chronic giardiasis can have serious effects on growth and development due to malnutrition (42). However, many of the asymptomatic or chronic patients can pass cysts to household members. The severity of the infection depends on virulence of the organism and the nutritional, immunological, and developmental status of the infected individual (43).

There are approximately 300 million estimated cases of giardiasis annually (44, 45). In developing countries the disease is quite prevalent. Even in the United States, a study indicated that about 15% of the nondysentric outpatient pediatric diarrhea can be attributed to *Giardia* (42). Unfiltered recreational, spring, lake and well water can contain Giardia cysts and is a common cause of diarrhea in campers and backpackers.

**Current Therapeutic options**

Nitroimidazole derivatives such as metronidazole or tinidazole are the drug of choice and are quite effective for treatment. However, metronidazole has never been approved by Food and Drug Administration (FDA) in the United States for the treatment of giardiasis (46). Side effects for both drugs include metallic taste, nausea, headache, abdominal discomfort, and neurological side effects (47). Notheless, Tinidazole is considered to be a better drug because of its higher efficacy as a single-dose therapy and less common side effects (42). Both drugs are mutagenic in bacteria and are carcinogenic in mice, therefore FDA has listed them as a risk during pregnancy (48). Transfer of electrons from parasite ferredoxins reduces the nitro groups of the nitrosohydroxyl amino moiety of the nitroimidazoles. The reduced drug binds to the DNA and causes strand breaks (49). Nitrofurans compounds such as Furazolidone is also used for the treatment of *Giardia* infection (50). The compound is activated following reduction by NADH oxidase and causes DNA damage (51). Nitazoxanide is another nitroheterocyclic compound that exhibited promising results for the treatment of giardiasis in both children and in adults (52, 53).

**Future Perspectives**

Although the current choices for the treatment of giardiasis are effective, significant toxicity of the
available drugs is of concern. In search of new drugs for therapy, recently a series of benzimidazole-pentamidine hybrids have been synthesized. Few of these compounds have been shown to be active in submicromolar concentrations (54). Sulfur-containing amino acids, e.g., cysteine has an important role in G. intestinalis survival by maintaining the cellular redox balance and providing the sulfur atom for the Fe-S clusters (55, 56). Unique aspects of sulfur-containing amino acid metabolism can be targeted for identifying new class of inhibitors (57). The sensitivity of Giardia's growth to oxygen led to the notion that the parasite can only be grown in sealed containers and no high-throughput screening assay existed. Recently a high-throughput assay in 384-well format has been developed for screening of chemical libraries.

The 12 Mb genome of Giardia containing 6470 has been sequenced (58) that has led to identification of many unique features that can be exploited as targets for the development of new generation of therapeutics. The genome sequencing has revealed the existence of 149 druggable proteins (59). However, it is likely there will be many additional key proteins that can be targeted. For example, proteins involved in encystation of trophozoites, such as cyst wall proteins, can be ideal targets that would prevent spread of the disease.

References