HHS Public Access

Author manuscript

Curr Trop Med Rep. Author manuscript; available in PMC 2016 September 01.

Published in final edited form as:

Curr Trop Med Rep. 2015 September ; 2(3): 181–187. doi:10.1007/s40475-015-0056-9.

Treatment of Cryptosporidium: What We Know, Gaps, and the Way Forward

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Abstract

Cryptosporidiosis is increasingly recognized as an important global health concern. While initially reported in immunocompromised such as AIDS patients, cryptosporidiosis has now been documented as a major cause of childhood diarrhea and an important factor in childhood malnutrition. Currently, nitazoxanide is the only proven anti-parasitic treatment for *Cryptosporidium* infections. However, it is not effective in severely immunocompromised patients and there is limited data in infants. Immune reconstitution or decreased immunosuppression is critical to therapy in AIDS and transplant patients. This limitation of treatment options presents a major public health challenge given the important burden of disease. Repurposing of drugs developed for other indications and development of inhibitors for novel targets offer hope for improved therapies, but none have advanced to clinical studies.

Keywords

Cryptosporidium; Cryptosporidium parvum; Cryptosporidium hominis; cryptosporidiosis; nitazoxanide; paromomycin

Introduction

Cryptosporidium species are increasingly recognized as important enteric pathogens [1-3]. Cryptosporidiosis was initially recognized as a cause of diarrhea in compromised hosts. Shortly thereafter, zoonotic and waterborne transmission of the parasite was identified. Cryptosporidium is now considered one of the major causes of childhood diarrhea. In

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Compliance with Ethics Guidelines: Conflict of Interest: Hayley Sparks, Gayatri Nair, Alejandro Castellanos-Gonzalez, and declare that they have no conflict of interest. A. Clinton White Jr receives royalties from UpToDate and Harrison's Internal Medicine for chapters on a different subject.

Human and Animal Rights and Informed Consent: This article does not contain any studies with human subjects performed by any of the authors. With regard to the authors' research cited in this paper, all institutional and national guidelines for the care and use of laboratory animals were followed.

addition, *Cryptosporidium* has been documented as a key component of the vicious cycle of infection and malnutrition that are major contributors to childhood morbidity and mortality worldwide. The majority of human *Cryptosporidium* infections are attributed to two species: *C. hominis* and *C. parvum* [1, 2]. However, at least 13 other species may infect humans, [3-5]. Clinically, cryptosporidiosis causes watery diarrhea in healthy patients. In contrast to other causes, diarrhea caused by cryptosporidiosis tends to be more prolonged and can be chronic in compromised hosts, such as children with malnutrition.

Cryptosporidium parasites develop within the microvillus layer of intestinal epithelial cells, mainly found in the small intestines in immunocompetent hosts, but may be found throughout the GI tract and even the respiratory tract. Persistent infection is associated with villus atrophy, crypt hyperplasia, and variable increases in leucocytes in the lamina propria. The symptoms of watery diarrhea and malabsorption are thought to be related to sodium malabsorption, electrogenic chloride secretion, and increased intestinal permeability, and severity of disease correlates with altered intestinal permeability [6, 7]. These effects are likely mediated by the host response and neuropeptides such as substance P may be key contributors [8, 9]

The burden of disease caused by *Cryptosporidium* worldwide has been significantly underestimated. For example, only about 1% of the estimated 750,000 cases that occur annually in the US are reported [10, 11]. Historically, *Cryptosporidium* was thought of primarily as a cause of chronic diarrhea in AIDS and other immunocompromised patients. More recent data has shed light on the parasite's effect on children in resource poor areas. Older studies, using acid fast staining to identify the organisms, found *Cryptosporidium* in <5% of cases of childhood diarrhea. However, more recent studies using antigen and molecular assay have detected *Cryptosporidium* infection in 15-20% of all childhood diarrhea [1, 12]. In a multicenter study of childhood diarrhea in Sub-Saharan Africa and South Asia, *Cryptosporidium* was second to rotavirus as a cause of moderate-to-severe diarrhea in children two years of age and was a major cause of morbidity in the second year of life [13]. Subsequent studies using molecular methods demonstrated that even that study had under diagnosed cryptosporidiosis [14].

There are also chronic sequellae of *Cryptosporidium* infection. Lima et al showed that infection in children less than one year old was followed by recurrent diarrhea and growth stunting that continued for two years after the initial episode [15]. Follow-up studies demonstrated deficits in cognitive development and physical fitness when patients were examined 5 years later [16].

Cryptosporidiosis is more frequent and severe disease in children with malnutrition, including higher incidence of deaths [2, 17, 18]. Malnutrition predisposes patients to infection and creates a vicious infection-malnutrition cycle with long term consequences including cognitive impairment and stunting [19]. Using an animal model, that closely resembles the complex interaction between nutritional status and infection, Costa *et al*. showed 20% additional weight loss when malnourished mice were infected with *C. parvum*, higher fecal shedding and failure to prevent weight loss or parasite stool shedding despite treatment with nitazoxanide [20]. In addition, children under the age of one year with

Cryptosporidium infections fail to have catch-up growth that is typically observed with children infected at a later age. The diarrheal morbidity for this young age group is significantly increased as well [18, 21].

Symptomatic therapy is vital in cases of cryptosporidiosis. Replacement of fluids and electrolytes in cryptosporidiosis is critically important as in other causes of diarrhea. Anti-motility drugs are also a key element of therapy. Most published patient studies utilize narcotic agents such as loperamide and diphenoxylate/atropine. Other reports suggest that tincture of opium (paregoric) may be a more effective agent in AIDS patients. Nutritional support is also imperative for successful treatment, which includes continued breast-feeding of infant patients.

Because cryptosporidiosis is typically self-limited in immunocompetent hosts, restoration of immune function is a key component of management. Immune reconstitution in response to effective combination antiretroviral therapy has been linked to parasite clearance, as well as reduced long term morbidity and mortality associated with cryptosporidial infection of AIDS patients [2, 22, 23]. Nevertheless, even with effective antiretroviral therapy, chronic diarrhea is associated with early mortality [24]. There are anecdotes of better responses when anti-motility and anti-parasitic drugs are used as part of the initial therapy [23]. However there is no conclusive evidence that this is the case. Interestingly, some HIV protease inhibitors have activity against *Cryptosporidium* both *in vitro* and *in vivo* [25].

Current Therapeutics

Despite the fact that cryptosporidiosis has been recognized as an important cause of diarrheal disease for over 3 decades, anti-parasitic treatments has been limited. Initial screening of available compounds failed to identify effective treatments for cryptosporidiosis. A number of drugs previously reported to be effective have failed in clinical trials [22].

The only drug that has FDA approval for treatment of *Cryptosporidium* is nitazoxanide [2]. Nitazoxanide was synthesized in the 1980s by combining a thiazole ring (similar structurally to metronidazole) with a benzamidine ring (similar to the tapeworm drug niclosamide). Nitazoxanide is a broad spectrum anti-parasitic with reports of use as a deworming agent as well as in controlled trials in giardiasis and cryptosporidiosis [22]. Three placebo-controlled trials of treatment of cryptosporidiosis with nitazoxanide in non-AIDS patients have been reported [26-28]. Studies reported up to 93% of treated patients experienced parasite clearance as opposed to 37% of placebo treated patients [27]. The drug also has been shown to improve diarrhea and mortality rates among infected, malnourished children [26]. However, the response rate in malnourished children was only 56% [26]. Unfortunately, nitazoxanide has not been found to be effective in AIDS patients [29].

Nitazoxanide is only FDA approved for patients one year of age or older. A recent study among hospitalized children in Egypt aged 6 months to 10 years presenting with persistent diarrhea compared paromomycin and nitazoxanide with no antiparasitic treatment. [30]. Overall, 86.6% of children treated with 100 or 200 mg of nitazoxanide every twelve hours for three days demonstrated complete clearance of oocysts and cessation of clinical

symptoms. Among children treated with 25 mg/kg/day of paromomycin for two weeks, 68.8% experienced stool clearance and were completely cured. Both treatments were better than no anti-parasitic treatment.

Though AIDS patients are the main immunocompromised population effected by *Cryptosporidium*, the parasite is also problematic in organ transplant recipients as well [31-33]. Bhadauria *et al.* conducted a retrospective review of living donor renal transplant recipients admitted for evaluation of diarrhea [33]. *Cryptosporidium* was found to be the most common cause of infectious diarrhea in these patients. Patients receiving combination immunosuppression including tacrolimus had higher rates of infection with *Cryptosporidium* compared to a combination of cyclosporine. Bhadauria and colleagues reported a better response to nitazoxanide/flouroquinolone combination therapy than to nitazoxanide alone [33]. However, the authors did not test the patients for *E. coli* enteropathogens. Thus, the benefit of the fluoroquinolone may have been on undiagnosed bacterial co-infection. Other groups have noted that transplant patients responded poorly to nitazoxanide alone. Still, there are anecdotes of better responses to combinations of nitazoxanide, azithromycin, and/or paromomycin [34-36].

Another population effected by chronic *Cryptosporidium* infections includes patients with primary immunodeficiencies such as hyper-IgM syndrome. This syndrome is a combined immune deficiency disorder caused by mutations in CD40 ligand. Infections in these patients are similar to that of AIDS patients, with extra-intestinal manifestations including biliary involvement. Fan *et al.*, showed that treatments with CD40 agonist antibody effectively reduced the number of oocytes shed by these patients. Although relapse occurred treatment was stopped [37].

Physicians initially approached the devastating effects of cryptosporidiosis in AIDS patients by testing a wide range of available drugs. One such drug, paromomycin, was reported to ameliorate cryptosporidiosis in AIDS patients [38]. In neither of the two controlled trials in patients with AIDS were the effects more than modest (few cures and mild decrease in diarrhea) [38, 39]. In one controlled trial, there was a statistically significant decrease in oocyst shedding and diarrhea [40]. While Hussien *et al.* demonstrated clearance of cryptosporidiosis among hospitalized children with paromomycin treatment compared to no treatment. However, the response was significantly less active than with nitazoxanide [30].

Other drugs reported to have some effect in case series include azithromycin, spiramycin, and bovine anti-cryptosporidium immunoglobulin [22]. However, all were ineffective in controlled trials in AIDS patients. Unfortunately, while all of these studies were presented at scientific meetings, none of these trials have been published [22]. Azithromycin seemed to be better than two anthelminthic drugs for cryptosporidiosis in children [41]. It has also been used in combination with nitazoxanide and/or paromomycin in compromised hosts with decreased stool frequency and parasite clearance in some patients [34, 42].

Rifamycins have been studied for anti-Cryptosporidium activity. Rifabutin, was tested *in vitro* and showed a 25% decrease in *C. parvum* infection in vitro. When combined with nitazoxanide, infection decreased by 75% [43]. Holmberg *et al.* [44] reported an 85%

decrease in the incidence of *Cryptosporidium* in the cohort of AIDS patients receiving rifabutin for *M. avium* complex prophylaxis, compared to the groups receiving clarithromycin or azithromycin. Similar results were seen when comparing AIDS patients taking rifabutin, clarithromycin or a combination [45]. A small group of HIV patients were found to have resolution of diarrhea with rifaximin, a non-absorbed rifamycin [46, 47].

Gaps

While nitazoxanide was an important advance in the management of cryptosporidiosis in children, its limited efficacy in compromised or malnourished hosts has raised important questions regarding how to manage these patients. Clearly, we are in urgent need of better drugs for therapy of cryptosporidiosis. A pivotal step towards this goal is the identification of specific targets. At an experts' workshop on cryptosporidiosis, development of novel drugs for cryptosporidiosis was considered a critically important priority, with potentially huge public health payoffs. The inability to propagate the organisms in vitro or to genetically manipulate parasite gene expression were identified as major hurdles for drug development [2].

Progress in developing novel drugs against *Cryptosporidium* also has been hampered by limitations of current experimental models [48]. Because animal models are suboptimal for some human infections, human cell lines have been used as an alternative to study intestinal pathogens [49, 50]. However the utility of cell lines is limited by the fact they do not readily support parasite propagation. However, novel methods incorporating intestinal stem cells offer the prospect of significantly improving propagation[51].

Further hindrances in the research and treatment of *Cryptosporidium* are the gaps in understanding of the gastrointestinal and immune responses to the parasite. There is a specific lack of knowledge regarding the mechanisms of parasite clearance in immunocompetent hosts. Insight into this aspect of infection might enable advances in preventative research. A more in-depth knowledge of the gastrointestinal responses is also needed to facilitate optimization of current treatment methods as well as provide specific targets for preventatives.

The Way Forward

One of the typical difficulties faced when developing anti-parasitic treatments is lack of drug targets that do not have human homologues. Several key enzymes have been identified as targets due to significant differences from human enzymes. Many apicomplexan parasites have unique calcium-dependent protein kinases, including the *Cryptosporidium* CDPK1, an essential component of cell invasion [52]. The parasites lack amino acids blocking access to the active site by “bumped kinase inhibitors”. Castellanos *et al.* has identified several compounds that bind to this enzyme, inhibiting enzyme function and ultimately killing the *Cryptosporidium* cell. A number of these inhibitors have exhibited anti-cryptosporidium activity both *in vitro* as well as in SCID-beige immunocompromised mouse models [53].

Clan CA cysteine proteases have been found to be a potential target for the treatment of cryptosporidiosis. Clan CA cysteine proteases are thought to be vital for host cell invasion

and has been found to be structurally different than analogous enzymes in humans [54]. This protease can be inhibited utilizing N-methyl-piperazine-Phe-homoPhe-vinylsulfone phenyl (K11777) inhibits growth of *Cryptospordium in vitro* and has been shown to rescue immunocompromised mice from lethal infection [55].

The folate biosynthesis pathway, historically a target for cancer, bacterial, and malarial disease has also been identified as a potential target for anti-cryptosporidials. *Cryptosporidium* contains a bi-functional thymidylate synthase/dihydrofolate reductase enzyme. Licensed anti-bacterial and anti-protozoan drugs do not inhibit the cryptosporidium enzyme but, research teams are evaluating the activity of compounds designed to specifically block this key enzyme in the folate synthesis pathway *in vitro.* Published data has reported striking anti-cryptosporidial activity of these compounds within cell culture [56].

Another potential drug target is oxidoreductase inosine 5′-monophosphate dehydrogenase (IMPDH) which is essential in guanine synthesis in *Cryptosporidium.* Unlike human IMPDH, *Cp*IMPDH seems to have originated from bacteria via lateral gene transfer thus the enzyme is structurally different than its human counterpart. Current research has found a series of inhibitors for this enzyme [57, 58]. Another study investigated the use of Phylomer® peptides to inhibit *Cp*IMPDH functions. These studies were conducted *in vitro* and identified two out of twelve peptides with anti-cryptosporidium functions [59].

The repurposing of already developed drugs or compounds is another promising area of anti-cryptosporidial research. A study done by Bessoff *et al.* utilized Human HMG-CoA Reductase and Isoprenoid Synthesis inhibitors from the NIH Clinical Collections drug library in a novel cell-based assay [60]. Results showed that there may be a surprising amount of overlap between previously FDA approved drugs and potential anti-cryptosporidal activity [60]. The use of Malaria Box drug-like compounds is also being investigated. These compounds have been created by the Medicines for Malaria Venture and are freely available to researchers [61]. A preliminary study identified several potential anti-cryptosporidiosis candidates however, further *in vitro* and *in vivo* research has yet to be published [62]. Similarly, the anti-inflammatory drug auranofin demonstrates activity against *Cryptosporidium in vitro* however, no *in vivo* or clinical results have been published [63].

The re-evaluation of current oral rehydration therapies is also being conducted to determine its role in the clearance of *Cryptosporidium* in immunocompetent hosts. Castro *et al.* demonstrated that supplementation of L-arginine to infected, undernourished mice aided in weight gain and reduced parasite burden [64]. Similar findings were reported by Costa *et al.* and Argenzio *et al.* utilizing the supplementation of an oligodeoxynucleotide with unmethylated CpG motif, alanyl-glutamine, and tumor necrosis factor alpha in *in vitro* and *in vivo* models [65, 66]. These findings indicate that current recommended oral rehydration therapies may need to be updated, however no clinical trials have been completed to asses these supplements in humans. Further research in this area could potentially provide a cost effective treatment option for malnourished and immunocompromised patients in resource-limited areas.

Conclusions

Within the past few years, studies have increasingly focused on the importance of *Cryptosporidium* as a cause of childhood diarrhea and associated morbidity and mortality. However, there has been little clinical advancement in the treatment of cryptosporidiosis. The study conducted by Hussien *et al.* concluded that nitazoxanide treatment is superior to paromomycin in children with chronic diarrhea in endemic areas [30]. Successful development of novel drugs could also aid in decreasing childhood malnutrition. Progress has been slowed by limitation in methods to propagate the organisms in vitro and genetically manipulate the organisms. Nevertheless, research focused on anti-cryptosporidials continues to make substantial advancements. Several enzymes have been identified as potential drug targets, including calcium-dependent protein kinases [53], Clan CA cysteine proteases [55], IMPDH [57] and the folate biosynthesis pathway [56]. Other studies are testing compounds repurposed, which were developed for other indications. However, none of these compounds have made it into clinical trials.

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Table 1
Current and prospective therapeutic options for Cryptosporidium infection

	Therapeutics	Research Level	Comments
Current Therapeutics	Nitazoxanide	FDA approved for treatment of patients 1 year and older	Not useful in patients with AIDS
	Nitazoxanide/ Azithromycin/ Paromomycin mixtures	Anecdotal clinical evidence of response in transplant patients	
	CD40 Agonist Antibody	One clinical study published	Patients relapsed after cessation of treatment
	Paromomycin	Multiple clinical studies	Clinical results have been mixed or moderate at best
	Azithromycin	One published clinical study	Decreases parasite load with clearance in some patients
	Rifamycins (Rifabutin, Rifaximin)	Several clinical studies which included AIDS patients	Prevented infection in AIDS patients
Prospective Therapeutics	CDPK Inhibitors	Several published *in vitro* and *in vivo* studies	
	N-methyl-piperazine-Phe-homoPhe-vinylsulfone phenyl (K11777)	Published *in vivo* studies	Inhibits Clan CA cysteine proteases
	Phylomer® Peptides	Published *in vitro* studies	Inhibits *Cp*IMPDH
	Compounds from NIH Clinical Collections	Published *in vitro* studies	Two compounds from this collection have been tested
	Malaria Box Drug-Like Compounds	One published *in vitro* study	
	Auranofin and other “orphan drugs”	Several published *in vitro* studies	Auranofin has been successfully tested against other apicomplexans *in vivo*

Note: Dr. White determined the Mechanisms column should not be included due to lack of information in this area.