**Summary:**
Cryptosporidiosis emergence triggered the screening of many compounds for potential anticyryptosporidial activity in which the majority were ineffective. The outbreak of cryptosporidiosis which occurred in Milwaukee in 1993 was not only the first significant emergence of Cryptosporidium spp. as a major human pathogen but also a huge waterborne outbreak thickening thousands of people from a major city in North America. Since then, outbreaks of cryptosporidiosis are regularly occurring throughout the world. New drugs against this parasite became consequently urgently needed. Among the most commonly used treatments against cryptosporidiosis are paromomycin, and azithromycin, which are partially effective. Nitazoxanide (NTZ)’s effectiveness was demonstrated in vitro, and in vivo using several animal models and finally in clinical trials. It significantly shortened the duration of diarrhea and decreased mortality in adults and in malnourished children. NTZ is not effective without an appropriate immune response. In AIDS patients, combination therapy restoring immunity along with antimicrobial treatment of Cryptosporidium infection is necessary. Recent investigations focused on the potential of molecular-based immunotherapy against this parasite. Others tested the effects of probiotic bacteria, but were unable to demonstrate eradication of C. parvum. New synthetic isoflavone derivatives demonstrated excellent activity against C. parvum in vitro and in a gerbil model of infection. Newly synthesized nitro or non nitro-thiazolide compounds, derived from NTZ, have been recently shown to be at least as effective as NTZ against C. parvum in vitro development and are promising new therapeutic agents.

**KEY WORDS:** cryptosporidiosis, nitazoxanide, thiazolides, EGF-R inhibitor, AIDS

Cryptosporidium is classified by the Centers for Disease Control and Prevention as an emerging infectious pathogen (Guerrant, 1997). Initially, C. parvum was believed to be only an occupational hazard of people exposed to infected animals, but it is now known to cause illness or disease both in immunocompetent and immunocompromised people. Cryptosporidiosis is one of the most common and certainly the most devastating gastrointestinal infection in people with AIDS. Outbreaks have occurred with contaminated water supplies most notably in 1993 when a waterborne outbreak in Milwaukee, Wisconsin infected over 400,000 people (Mac Kenzie, 1994). Most human infections are thought to be caused by C. parvum or C. hominis although other Cryptosporidium species are reported to cause human infection, particularly in immunocompromised individuals (Flynn, 2003; Caccio, 2002). The propensity of the parasite to survive and be transmitted through source waters makes this an important public health threat.

The life cycle and pathophysiology of Cryptosporidium have been well-described (Tzipori, 2000). The end-result of infection is epithelial cell death leading to villous atrophy, malabsorption, and altered intestinal permeability, all of which contributes to the voluminous watery diarrhea seen in acute infection. Immunocompetent individuals have a self-limited illness and presentation of disease in immunocompromised patients depends on the level of immunosuppression. Much of what is known about the clinical features of cryptosporidiosis in immunocompromised hosts has been learned from patients with HIV infection. In patients with AIDS, cryptosporidiosis is self-limited in individuals with CD4 cells count higher than 180 cells per cu.mm, chronic in patients with CD4 cell depletion to less than 100 cells per cu.mm, and fulminant in some of those with count below 50 cells per cu.mm. (Colford, 1996).

With the numbers of immunosuppressed patients from transplantation or chemotherapy increasing, cryptosporidiosis become an increasingly common problem and on a worldwide scale in malnourished infants and children.

**DRUGS WITH ANTICRYPTOSPORIDIAL ACTIVITY**

**Paromomycin**

Paromomycin is an aminoglycoside antibiotic poorly absorbed from the gut epithelium when given orally but apparently, it can be absorbed in small quantities across the limiting apical membrane.
bunding the extracytoplasmic parasite. Its mechanism of action is targeting the bacterial ribosome where it disrupts protein synthesis. Based on experimental data and clinical experience, paromomycin has a modest activity against *C. parvum* (Griffith, 1998). Four small and two large open uncontrolled studies have evaluated the efficacy of this drug prospectively. In most studies there was a good clinical and parasitological response. However, after paromomycin discontinuation many patients relapsed (Giezy, 1991; Armitage, 1992; Fichtenbaum, 1993; Wallace, 1993; Bissuel, 1994), moreover a meta-analysis of data from several studies performed from 1990 to 1996 in immunocompromised patients concluded that it was only partially effective (Abubakar, 2007). Well controlled clinical studies are limited. Hewitt et al. in 2000 conducted a prospective, randomized, double-blind, placebo-controlled study to evaluate the efficacy of paromomycin in the treatment of symptomatic cryptosporidial enteritis in AIDS patients and paromomycin was not shown to be more effective than placebo. White et al. in 1994 performed a double-blind clinical study in ten patients with AIDS and cryptosporidiosis, showed that oocyst excretion decreased significantly and suggested that patients should receive maintenance therapy to prevent relapse.

**Azithromycin**

In animal models, azithromycin, an azalide antibiotic interfering with the microbial protein synthesis, is the most active among the macrolides against cryptosporidiosis in animal models (Rehg, 1994). Kadappu et al. (2002) performed a randomized, controlled trial to study the efficacy of short-term use of azithromycin in thirteen AIDS patients. There was a good clinical improvement but parasitological benefit was doubtful. Dionsio et al., 1998 reported that “long term, low dose azithromycin is well tolerated and may induce stable remission of chronic cryptosporidiosis in patients with AIDS. It may lead to probable eradication of the infection in some patients, even those with severe immunodeficiency”. When given in combination with paromomycin, good results were reported with azithromycin in a small open-label trial with 11 HIV patients with cryptosporidiosis (Smith, 1998).

**Roxithromycin**

Another macrolide, roxithromycin, is also being studied as a possible treatment option for cryptosporidiosis but clinical data are extremely limited. Efficacy of roxithromycin was investigated in 26 patients with AIDS, 22 of whom completed the study, 15 patients (68.2 %) were cured, six (27.3 %) improved, and treatment failed in one patient (Uip, 1998). Additional controlled clinical trials are necessary.

**Nitazoxanide**

Nitazoxanide (NTZ), first-in-class thiazolide is a 5-nitrothiazolyl salicylamide derivative with broad activity against protozoa and helminths. NTZ and its two metabolites, tizoxanide (TZ) and tizoxanide-glucuronide (TZglu) were shown to inhibit the growth of *C. parvum* at concentrations lower than 10 mg/L (Theodos, 1998; Gargala, 2000; Cai, 2005). In vivo, it was subsequently found to be effective against *C. parvum* in suckling mice, nude mice, gerbils, rats and piglets (Theodos, 1998; Blagburn, 1998; Li, 2003; Baishanbo, 2005). TZ had only limited activity, but NTZ and TZglu strongly inhibited asexual and sexual stages, respectively (Gargala, 2000). NTZ was shown to be more effective than paromomycin on biliary tract cryptosporidiosis in immunosuppressed gerbils (Baishanbo, 2006). The efficacy of NTZ in treating cryptosporidiosis in immunocompetent patients was well established by three double blind placebo-controlled clinical studies carried out in more than 140 immunocompetent adults and 150 immunocompetent children from Egypt and Zambia (Rossignol, 2001, 2006; Amadi, 2002). Clinical and parasitological cures were recorded following a three-day course of treatment in adults, adolescents and children (1-11 years of age). In addition, there was a statistically significant reduction of malnourished infants mortality. Two double-blind placebo controlled studies of NTZ in the treatment of cryptosporidial diarrhea in adult AIDS patients were carried out in 66 Mexicans and 50 Thais. The drug was given as 1,000 mg twice a day for two weeks in patients with CD-4 counts > 50 cells per cu.mm and eight weeks in patients with CD-4 counts < 50 cells per cu.mm. The drug reached statistical significance (P < 0.05) in both clinical resolution of diarrhea and suppression of the oocyst shedding (Rossignol, 1998, 2006). Three hundred and sixty-five (365) patients were enrolled at 165 study centers throughout the USA. The duration of treatment ranged from one to 1,528 days (median 62 days). Among the 357 patients included in the intent to treat analysis, 209 (59 %) achieved a sustained clinical response while on treatment. Clinical responses were closely associated with *C. parvum* negative stools (P < 0.0001). No safety issues were identified at doses up to 3,000 mg/day or for long duration of treatment (Rossignol, 2006). NTZ and TZ account for activity in the intestine, while in other locations TZglu is the most important agent (i.e. bile). High concentrations of TZglu are excreted in bile and TZglu is believed to be responsible for the activity of the compound in cholangitis produced by *C. parvum* in disseminated cryptosporidiosis in immunocompromised subjects. A systematic review and meta-analysis was carried out up to August 2005 to assess the efficacy of interventions for the treatment and prevention of cryptosporidiosis among immunocompromised patients. The results indicate that
NTZ reduces load of parasites and may be useful in immunocompetent individuals (Abubakar, 2007). De La Tribonnière et al. (1999) reported a systemic cryptosporidiosis in a 51-year old man with AIDS, with sclerosing cholangitis, resolutive with oral NTZ (500 mg of NTZ bid for 12 weeks) and paromomycin inhalation for 15 days because of C. parvum detection in stools and bronchoalveolar lavage. Triple antiretroviral therapy was added and the CD4 increased from 38 to 70 cells/mm³. One year later, the CD4 dropped to 12 and the cholangitis reappeared. C. parvum was isolated in stools and NTZ therapy was reintroduced and the cholestasis again decreased. Faraci et al. (2007) described a case of Cryptosporidium (intestinal, biliary and pancreatic) infection occurring in a child after allogeneic stem cell transplantation (SCT) for acute non-lymphoblastic leukemia. The increase in CD3+CD4+ cells secondary to the reduction of steroid therapy associated with the improvement of intestinal acute graft versus host disease and the use of NTZ improved the infection-related symptoms and led to a complete clearance of Cryptosporidium.

OTHER ANTIMICROBIAL AGENTS

Octreotide acetate, atovaquone, letrazuril, and lasolacid have been investigated in immunosuppressed persons but with poor efficacy and inconsistent results (Zardi, 2005).

OTHER THERAPIES

ANTIRETROVIRAL THERAPY

The most effective strategy for managing Cryptosporidium in HIV-infected patients appears to be immune reconstitution with highly active antiretroviral therapy (Miao, 2000). Some studies using protease inhibitors such as ritonavir, saquinavir, and indinavir claim a drastic reduction of C. parvum infection both in vivo and in vitro (Mele, 2003; Hommer, 2003). Whether or not aspartyl proteases could have some important functions is not known as there are not reports of its presence in C. parvum. While HAART should increase patient’s CD4 above risk thresholds, concomitant target of the opportunistic infection remain important to prevent on going morbidity.

ANTIBODY THERAPY

A few case reports have suggested that hyperimmune bovine colostrum may modify the disease course of Cryptosporidium infection in immunocompromised hosts (Greenberg, 1996; Nord, 1990). Results obtained from bovine colostrum antibody therapy are mostly contradictory. Other therapies include passive immunization via human breast milk or oral human immunoglobulin (Borowitz, 1991; Kuhls, 1995; Pape, 1987). None of these therapies have been evaluated in randomized, placebo-controlled trials (Crabb, 1998). Riggs et al. in 2002, in a work regarding neutralizing monoclonal antibodies (MAbs), hypothesized that targeting the apical complex and surface antigens CSL, GP25-200, and P23 could passively immunize against cryptosporidiosis. The results indicated that anti-CSL MAB 3E2 had highly significant efficacy in reducing, but not eliminating, persistent C. parvum infection in adult gamma interferon-depleted SCID mice.

PROBIOTICS

Experimental study of the effects of probiotics on Cryptosporidium infection in neonatal rats showed a trend to a more rapid clearance of parasites in rats treated with probiotics. Overall, results showed that the daily administration of L. casei-containing mixtures was unable to eradicate the parasite in neonatal rats model (Guitard, 2006).

NUTRITIONAL REHABILITATION

Aggressive rehydration and nutritional support with total parenteral nutrition may be necessary due to massive stool losses sometimes in excess of 2 L. Glutamine or alanyl glutamine has been used in patients with severe diarrhea to aid in fluid and electrolyte absorption. In animal models of Cryptosporidium and Rotavirus, glutamine has been shown to stimulate sodium reabsorption in the small intestine even in the context of bowel villous atrophy (Blikslager, 2001).

RECENT ADVANCES IN DRUG DISCOVERY AND NOVEL DRUG TARGETS

NEW THIAZOLIDE COMPOUNDS

Forty two new thiazolide compounds, NTZ-derivatives, have been synthesized by the Romark Center for Drug Discovery. Modifications of the NTZ chemical structure have been performed either on the nitro group containing thiazole moiety either on the benzene moiety. 26 out of 42 thiazolides exerted a ≥ 95 % inhibition against C. parvum in vitro development. Fifteen agents have been found to have lower IC50 than NTZ (1.2 mg/L for NTZ). All thiazolides with a chloro- instead a nitro-group exerted > 95 % inhibition and most bromo-thiazolides exerted > 95 % inhibition. Anticyptosporidial activity is thought to be independent of the presence of a nitro group on the thiazole moiety. This means that, in such compounds, the nitro-group could be eliminated, and some of the chloro- and/or bromo-thiazolides could represent a valua-
ble alternative to the parent compound. While de-ace-
ylated compounds (primary metabolic products) exhi-
bited largely similar activities than their respective ace-
ylated homolog, these new thiazolides are thought to
be effective in vivo and are promising drug candidates
(Gargala, submitted).

MODE OF ACTION OF THIAZOLIDES
ON INTRACELLULAR PROTOZOA

Based on studies on anaerobic bacteria, NTZ has been
postulated to exhibit a mode of action related to the
functional inhibition of pyruvate ferredoxin oxi-
ductase (PFOR). There are most likely fundamental dif-
fferences with regard to the mode of action of NTZ and
NTZ-derivatives between anaerobic bacteria, and intra-
acellular and extracellular parasites (Hemphill, 2006;
Müller, 2007). Data obtained from studies on Giardia
and Neospora caninum, an intracellular Apicomplexa,
suggests a model for the mode of action of thiazolides
in which, both the thiazole moiety with or without
nitro-group, as well as the benzene moiety of the mole-
cule, determine host specificity and efficacy (Esposito,
2005, 2007a, b; Müller, 2006). Protein disulfide isome-
rase is an important endoplasmic reticulum (ER)-asso-
ciated enzyme involved in maintaining the correct three-
dimensional structure of polypeptides, and synthesis
during synthesis in the ER. In vitro studies on Neospora
have shown that the enzyme activity of recombinant NcPDI
is inhibited by NTZ and other nitro and non-nitrothiazolides
that also inhibit parasite proliferation. Inhibition of
NcPDI could result in an impairment of its functions,
leading to protein misfolding followed by an impaired
metabolic activity (Naguleswaran, 2005; Müller 2007).

ISOFLAVONE DERIVATIVES

In 1987, Akiyama et al. described the tyrosine-specific
protein kinase activity of the epidermal growth factor
(EGF) receptor, pp60v-src and pp110gag-fes that was
inhibited in vitro by the isoflavone genistein. In 2006,
Stachulski et al. synthesized 52 EGFR-PTK inhibitor iso-
flavone analogs such as dihydroxyisoflavone and tri-
hydroxydeoxybenzene derivatives. For identifying anti-
coccidial agents among these new agents, Gargala et
al. (2006) studied the activities of EGF receptor inhibi-
tors against Sarcocystis neurona, Neospora caninum,
and C. parvum grown in cell cultures. Agents induced
a maximum development inhibition (MI) of more than
95 % for at least one parasite. For five agents, the MI
was ≥ 95 % for the three parasites. For two isoflavone
derivatives compounds, excellent activity against C. par-
vum was observed not only in cell cultures but also in a C. parvum-infected immunosuppressed gerbil model.
The in vitro activity of these two isoflavones (RM-6427
and RM-6428) were quite effective not only on intes-
tinal but also on biliary cryptosporidiosis.

BISPHOSPHONATES AS CHEMOTHERAPEUTIC AGENTS
AGAINST Cryptosporidium

Apicomplexan parasites including C. parvum are known
to utilize pyrophosphate in place of ATP as an energy
donor by the action of a pyrophosphate dependent
phosphofructokinase (PF-PFK) and also have a far-
nesyl pyrophosphate synthase. (Vedadi, 2007). Moreno
et al. in 2001 have reported that nonhydrolyzable
pyrophosphate analogs, bisphosphonates and especially
risedronate, currently used in bone resorption therapy
and known to be potent inhibitors of the enzyme far-
nesyl pyrophosphate synthase, inhibit the growth of
C. parvum in a mouse xenograft model. Millions of peo-
ple have been treated to date with bisphosphonates
and since they are already FDA-approved they consti-
tute an attractive group to develop as chemotherapeutic
agents against protozoal diseases.

POLYamine METABOLISM
AS CHEMOTHERAPEUTic TARGET

Sinefungin has a well documented high anti-
cryptosporidial activity, in vitro and in an immunosuppressed rat
model of C. parvum infection and the biochemical
mechanism of its antiparasitic activity was thought to
involve transmethylation reactions or enzymes of poly-
amine biosynthesis (Lemeteil, 1993; Gargala, 1999). The
polyamines spermidine and spermine are cationic
molecules that have several indispensable cellular func-
tions (Bacchi, 2002). However, initiation of polyamine
synthesis in C. parvum occurs via a pathway most com-
monly used by plants and certain bacteria. The discovery
of a highly divergent spermidine/spermine N1-acetyl-
transferase (SSAT) that mediates a reverse polyamine
biosynthetic pathway in C. parvum and that is selecti-
vely blocked by conformationally restricted polyamine
analogues provides a selective target for the design of
anti-cryptosporidial compounds (Yarlett, 2007a, b).

CONCLUSION

NTZ alone or perhaps combined with paromomycin could be used for treating cryptospo-
diosis. There is little doubt that more antipro-
zoal drugs are needed. Compounds targeting the EGF
receptor, pp60v-src and pp110gag-fes could be effec-
tive against Cryptosporidium spp. while new thiazolide
compounds active against protein disulfide isomerases
more specifically PD12 and PD14 as recently reported
(Muller, 2007) could also represent a promising targets
for new drugs effective against Cryptosporidium spp.
Bisphophonates and polyamine analogs could undoub-
tedly represent new options, based on selective targets,
for the design of anticyclosporidial agents.
REFERENCES


