Article available at http://www.parasite-journal.org or http://dx.doi.org/10.1051/parasite/2008153275

DRUG TREATMENT AND NOVEL DRUG TARGET AGAINST *CRYPTOSPORIDIUM*

GARGALA G.*

Summary:

Cryptosporidiosis emergence triggered the screening of many compounds for potential anti-cryptosporidial 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

* Laboratory of Parasitology, ADEN EA-3234, Faculty of Medicine and Pharmacy, Rouen University, 22, Boulevard Gambetta, F-76183 Rouen Cedex France.
Tel: 33 (0) 2 35 14 85 79 – Fax: 33 (0)2 32 88 66 75.
E-mail:gilles.gargala@univ-rouen.fr

bounding 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 (Ciezy, 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. Dionisio *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 31-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/mm^3 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. Anticryptosporidial 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-acetylated compounds (primary metabolic products) exhibited largely similar activities than their respective acetylated 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 oxidoreductase (PFOR). There are most likely fundamental differences with regard to the mode of action of NTZ and NTZ-derivatives between anaerobic bacteria, and intracellular 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 molecule, determine host specificity and efficacy (Esposito, 2005, 2007a, b; Müller, 2006). Protein disulfide isomerase is an important endoplasmic reticulum (ER)-associated enzyme involved in maintaining the correct three-dimensional structure of polypeptides 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 isoflavone analogs such as dihydroxyisoflavone and trihydroxydeoxybenzoine derivatives. For identifying anticoccidial agents among these new agents, Gargala *et al.* (2006) studied the activities of EGF receptor inhibitors 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. parvum* was observed not only in cell cultures but also in a *C. parvum*-infected immunosupressed gerbil model. The *in vivo* activity of these two isoflavones (RM-6427 and RM-6428) were quite effective not only on intestinal 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 (PPi-PFK) and also have a farnesyl 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 farnesyl pyrophosphate synthase, inhibit the growth of *C. parvum* in a mouse xenograft model. Millions of people have been treated to date with bisphosphonates and since they are already FDA-approved they constitute an attractive group to develop as chemotherapeutic agents against protozoal diseases.

Polyamine metabolism as chemotherapeutic target

Sinefungin has a well documented high anticryptosporidial 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 polyamine biosynthesis (Lemeteil, 1993; Gargala, 1999). The polyamines spermidine and spermine are cationic molecules that have several indispensable cellular functions (Bacchi, 2002). However, initiation of polyamine synthesis in *C. parvum* occurs via a pathway most commonly used by plants and certain bacteria. The discovery of a highly divergent spermidine/spermine N1-acetyltransferase (SSAT) that mediates a reverse polyamine biosynthetic pathway in *C. parvum* and that is selectively 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 cryptosporidiosis. There is little doubt that more antiprotozoal drugs are needed. Compounds targeting the EGF receptor, pp60v-src and pp110gag-fes could be effective against *Cryptosporidium* spp. while new thiazolide compounds active against protein disulfide isomerases more specifically PDI2 and PDI4 as recently reported (Muller, 2007) could also represent a promising targets for new drugs effective against *Cryptosporidium* spp. Biphosphonates and polyamine analogs could undoubtedly represent new options, based on selective targets, for the design of anticryptosporidial agents.

REFERENCES

ABUBAKAR I., ALIYU S.H., ARUMUGAM C., USMAN N.K. & HUNTER P.R. Treatment of cryptosporidiosis in immunocompromised individuals: systematic review and meta-analysis. *Br. J. Clin. Pharmacol.*, 2007, *63* (4), 387-393.

AKIYAMA T., ISHIDA J., NAKAGAWA S., OGAWARA H., WATANABE S., ITOH N., SHIBUYA M. & FUKAMI Y. Genistein, a specific inhibitor of tyrosine-specific protein kinases. *J. Biol. Chem.*, 1987, *262* (12), 5592-5595.

AMADI B., MWIYA M., MUSUKU J., WATUKA A., SIANONGO S., AYOUB A. & KELLY P. Effect of NTZ on morbidity and mortality in Zambian children with cryptosporidiosis: a randomised controlled trial. *Lancet*, 2002, *360* (9343), 1375-1380.

ARMITAGE K., FLANIGAN T., CAREY J., FRANK I., MACGREGOR R., ROSS P. GOODGAME R. & TURNER J. Treatment of cryptosporidios with paromomicyn. A report of five cases. *Arch. Intern. Med.*, 1992, *152* (12), 2497-2499.

BACCHI C.J. & YARLETT N. Polyamine metabolism as chemotherapeutic target in protozoan parasites. *Mini. Rev. Med. Chem.*, 2002, *2* (6), 553-563.

BAISHANBO A., GARGALA G., DUCLOS C., FRANÇOIS A., ROSSIGNOL J.F., BALLET J.J. & FAVENNEC L. Efficacy of nitazoxanide and paromomycin in biliary tract cryptosporidiosis in an immunosuppressed gerbil model. *J. Antimicrob. Chemother.*, 2006, *57* (2), 353-355.

BISSUEL F., COTTE L., RABODONIRINA M., ROUGIER P., PIENS M.A. & TREPO C. Paromomycin: an effective treatment for cryptosporidial diarrhea in patients with AIDS. *Clin. Infect. Dis.*, 1994, *18* (3), 447-449.

BLAGBURN B.L., DRAIN K.L., LAND T.M., KINARD R.G., MOORE P.H., LINDSAY D.S., PATRICK D.A., BOYKIN D.W. & TIDWELL R.R. Comparative efficacy evaluation of dicationic carbazole compounds, nitazoxanide and paromomycin against *C. parvum* infections in a neonatal mouse model. *Antimicrob. Agents Chemother.*, 1998, *42* (11), 2877-2882.

BLIKSLAGER A., HUNT E., GUERRANT R., RHOADS M. & ARGENZIO R. Glutamine transporter in crypts compensates for loss of villus absorption in bovine cryptosporidiosis. *Am. J. Physiol. Gastrointest. Liver. Physiol.*, 2001, *281* (3), G645-G653.

BOROWITZ S.M. & SAULSBURY F.T. Treatment of chronic cryptosporidial infection with orally administered human serum immune globulin. *J. Pediatr.*, 1991, *119* (4), 593-595.

CACCIO S., PINTER E., FANTINI R., MEZZAROMA I. & POZIO E. Human infection with *Cryptosporidium* felis: Case report and literature review. *Emerg. Infect. Dis.*, 2002, *8* (1), 85-86.

CAI X., WOODS K.M., UPTON S.J. & ZHU G. Application of quantitative real-time reverse transcription-PCR in assessing drug efficacy against the intracellular pathogen *Cryptosporidium parvum in vitro*. *Antimicrob. Agents Chemother.*, 2005, *49* (11), 4437-4442.

CIEZY K., GOLD J., BLAZE J. & JONES P. Paromomycin for the treatment of cryptosporidial diarrhoea in AIDS patients. *AIDS*, 1991, *5* (9), 1146-1147.

COLFORD J.M. JR., TAGER I.B., HIROZAWA A.M., LEMP G.F., ARAGON T. & PETERSEN C. Cryptosporidiosis among patients infected with human immunodeficiency virus. Factors related to symptomatic infection and survival. *Am. J. Epidemiol.*, 1996, *144* (9), 807-816.

CRABB J.H. Antibody-based immunotherapy of cryptosporidiosis. *Adv. Parasitol.*, 1998, *40*, 121-149.

DE LA TRIBONNIERE X., VALETTE M. & ALFANDARI S. Oral nitazoxanide and paromomycin inhalation for systemic cryptosporidiosis in a patient with AIDS. *Infection*, 1999, *27* (3), 232.

DIONISIO D., ORSI A., STERRANTINO G., MELI M., DI LOLLO S., IBBA MANNESCHI L., TROTTA M., POZZI M., SANI L. & LEONCINI F. Chronic cryptosoporidiosis in patients with AIDS: stable remission and possible eradication after long-term, low dose azithromycin. *J. Clin. Pathol.*, 1998, *51* (2), 138-142.

ESPOSITO M., MOORES S., NAGULESWARAN A., MÜLLER J. & HEMPHILL A. Induction of tachyzoite egress from cells infected with the protozoan *Neospora* caninum by nitro- and bromo-thiazolides, a class of broad-spectrum anti-parasitic drugs. *Int. J. Parasitol.*, 2007a, *37* (10), 1143-1152.

ESPOSITO M., MÜLLER N. & HEMPHILL A. Structure-activity relationships from *in vitro* efficacies of the thiazolide series against the intracellular apicomplexan protozoan *Neospora* caninum. *Int. J. Parasitol.*, 2007b, *37* (2), 183-190.

ESPOSITO M., STETTLER R., MOORES S.L., PIDATHALA C., MÜLLER N., STACHULSKI A., BERRY N.G., ROSSIGNOL JF. & HEMPHILL A. *In vitro* efficacies of nitazoxanide and other thiazolides against *Neospora* caninum tachyzoites reveal antiparasitic activity independent of the nitro group. *Antimicrob. Agents Chemother.*, 2005, *49* (9), 3715-3723.

FARACI M., CAPPELLI B., MORREALE, G., LANINO E., MORONI C., BANDETTINI M., TERRANOVA M.P., DI MARTINO D., COCCIA C & CASTAGNOLA E. Nitazoxanide or $CD3^+/CD4^+$ lymphocytes for recovery from severe *Cryptosporidium* infection after allogeneic bone marrow transplant? *Pediatr. Transplant.*, 2007, *11* (1), 113-116.

FICHTENBAUM C.J., RITCHIE D.J. & POWDERLY W.G. Use of paromomycin for treatment of *Cryptosporidium* in patients with AIDS. *Clin. Infect. Dis.*, 1993, *16* (2), 298-300.

FLYNN PM. *C. parvum*, *in*: Principles and practice of pediatric infectious diseases, 2nd edn. Long S.S., Pickering L.K., Prober C.G (eds), Philadelphia: Churchill Livingstone, 2003, 1267-1269.

GARGALA G., DELAUNAY A., FAVENNEC L., BRASSEUR P. & BALLET J.J. Enzyme immunoassay detection of *Cryptosporidium parvum* inhibition by sinefungin in sporozoite infected HCT-8 enterocytic cells. *Int. J. Parasitol.*, 1999, *29* (5), 703-709.

GARGALA G., DELAUNAY A., LI X., BRASSEUR PH., FAVENNEC L. & BALLET J.J. Efficacy of nitazoxanide, tizoxanide and tizoxanide glucuronide against *Cryptosporidium parvum* development in sporozoite-infected HCT-8 enterocytic cells. *J. Antimicrob. Chemother.*, 2000, *46* (1), 57-60.

GARGALA G., BAISHANBO A., FAVENNEC L., FRANÇOIS A., BALLET J.J. & ROSSIGNOL J.F. Inhibitory activities of epidermal growth factor receptor tyrosine kinase-targeted dihydroxyisoflavone and trihydroxydeoxybenzoin derivatives on Sarcocystis neurona, *Neospora* caninum and *C. parvum* development. *Antimicrob. Agents Chemother.*, 2005, *49* (11), 4628-4634.

GREENBERG P.D. & CELLO J.P. Treatment of severe diarrhea caused by *C. parvum* with oral bovine immunoglobulin concentrate in patients with AIDS. *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol.*, 1996, *13* (4), 348-354.

GRIFFITHS J.K., BALAKRISHNAN R., WIDMER G. & TZIPORI S. Paromomycin and genecitin inhibit intracellular *C. parvum* without trafficking through the host cell cytoplasm: implications for drug delivery. *Infect. Immun.*, 1998, *66* (8), 3874-3883.

Guerrant R.L. Cryptosporidiosis: An emerging, highly infectious threat. *Emerg. Infect. Dis.*, 1997, *3* (1), 51-57.

GUITARD J., MENOTTI J., DESVEAUX A., ALIMARDANI P., PORCHER R., DEROUIN F. & KAPEL N. Experimental study of the effects of probiotics on *C. parvum* infection in neonatal rats. *Parasitol. Res.*, 2006, *99* (5), 522-527.

HEMPHILL A., MUELLER J. & ESPOSITO M. Nitazoxanide, a broad-spectrum thiazolide anti-infective agent for the treatment of gastrointestinal infections. *Expert. Opin. Pharmacother.*, 2006, *7* (7), 953-964.

HEWITT R.G., YIANNOUTSOS C.T., HIGGS E.S. CAREY J.T., GEISELER P.J., SOAVE R., ROSENBERG R., VAZQUEZ G.J., WHEAT L.J., FASS R.J., ANTONINIEVIC Z., WALAWANDER A.L., FLANIGAN T.P. & BENDER J.F. Paromomycin: no more effective than placebo for treatment of cryptosporidiosis in patients with advanced human immunodeficiency virus infection. *Clin. Infect. Dis.*, 2000 , *31* (4), 1084-1092.

HOMMER V., EICHHOLZ J. & PETRY F. Effect of antiretroviral protease inhibitors alone, and in combination with paromomycin, on the excystation, invasion and *in vitro* development of *C. parvum*. *J. Antimicrob. Chemother.*, 2003, *52* (3), 359-364.

KADAPPU K.K., NAGARAJA M.V., RAO P.V. & SHASTRY B.A. Azithromycin as treatment for ceyprosporidiosis in human immunodeficiency virus disease. *J. Postgrad. Med.*, 2002, *48* (3), 179-181.

KUHLS T.L., ORLICEK S.L., MOSIER D.A., CRAWFORD D.L., ABRAMS V.L. & GREENFIELD R.A. Enteral human serum immunoglobulin treatment of cryptosporidiosis in mice with severe combined immunodeficiency. *Infect. Immun.*, 1995, *63* (9), 3582-3586.

LEMETEIL D., ROUSSEL F., FAVENNEC L., BALLET, J.J. & BRASSEUR P. Assessment of candidate anticryptosporidial agents in an immunosuppressed rat model. *J. Infect. Dis.*, 1993, *167* (3), 766-768.

LI X., BRASSEUR P., AGNAMEY P., LEMETEIL D., FAVENNEC L., BALLET J.J. & ROSSIGNOL J.F. Long-lasting anticryptosporidial activity of nitazoxanide in an immunosuppressed rat model. *Folia Parasitol. (Praha)*, 2003, *50* (1), 19-22.

MAC KENZIE W.R., HOXIE N.J., PROCTOR M.E., GRADUS M.S., BLAIR K.A., PETERSON D.E., KAZMIERCZAK J.J., ADDISS D.G., FOX K.R., ROSE J.B. & DAVIS J.P. A massive outbreak in Milwaukee of *Cryptosporidium* infection transmitted through the public water supply. *N. Engl. J. Med.*, 1994, *331* (3), 161-167.

MELE R., GOMEZ MORALES M.A., TOSINI F. & POZIO E. Indinavir reduces *C. parvum* infection in both *in vitro* and *in vivo* models. *Int. J. Parasitol.*, 2003, *33* (7), 757-764.

MIAO Y.M., AWAD-EL-KARIEM F.M., FRANZEN C., ELLIS D.S., MÜLLER A., COUNIHAN H.M., HAYES P.J. & GAZZARD B.G. Eradication of cryptosporidia and microsporidia following successful antiretroviral therapy. *J. Acquir. Immune Defic. Syndr.*, 2000, *25* (2), 124-129.

MORENO B., BAILEY B.N., LUO S., MARTIN M.B., KUHLENSCHMIDT M., MORENO S.N., DOCAMPO R. & OLDFIELD E. ^{31}P NMR of Apicomplexans and the effects of risedronate on *C. parvum Growth Biochem. Biophys. Res. Commun.*, 2001, *284* (3), 632-637.

MÜLLER J., NAGULESWARAN A., MÜLLER N. & HEMPHILL A. *Neospora caninum*: Functional inhibition of protein disulfide isomerase by the broad-spectrum anti-parasitic drug nitazoxanide and other thiazolides. *Exp. Parasitol.*, 2007, in press.

NAGULESWARAN A., ALAEDDINE F., GUIONAUD C., VONLAUFEN N., SONDA S., JENOE P., MEVISSEN M. & HEMPHILL A. *Neospora caninum* protein disulfide isomerase is involved in tachyzoite-host cell interaction. *Int. J. Parasitol.*, 2005, *35* (13), 1459-1472.

NORD J., MA P., DIJOHN D., TZIPORI S. & TACKET C.O. Treatment with bovine hyperimmune colostrum of cryptosporidial diarrhea in AIDS patients. *AIDS*, 1990, *4* (6), 581-584.

PAPE J.W., LEVINE E., BEAULIEU M.E., MARSHALL F., VERDIER R. & JOHNSON W.D. Jr. Cryptosporidiosis in Haitian children. *Am. J. Trop. Med. Hyg.*, 1987, *36* (2), 333-337.

REHG J.E. A comparison of anticryptosporidial activity of paromomycin with that of other aminoglycosides and azithromycin in immunosuppressed rats. *J. Infect. Dis.*, 1994, *170* (4), 934-938.

RIGGS M.W., SCHAEFER D.A., KAPIL S.J., BARLEY-MALONEY L. & PERRYMAN L.E. Efficacy of monoclonal antibodies against defined antigens for passive immunotherapy of chronic gastrointestinal cryptosporidiosis. *Antimicrob. Agents Chemother.*, 2002, *46* (2), 275-282.

ROSSIGNOL J.F., HIDALGO H., FEREGRINO M., HIGUERA F., GOMEZ W.H., ROMERO J.L., PADIERNA J., GEYNE A. & AYERS M.S. A double-blind placebo controlled study of nitazoxanide in the treatment of cryptosporidial diarrhea in AIDS patients. *Trans. R. Soc. Trop. Med. Hyg.*, 1998, *92* (6), 663-666.

ROSSIGNOL J.F., AYOUB A. & AYERS M.S. Treatment of diarrhea caused by *C. parvum*: a prospective randomized, double-blind, placebo-controlled study of nitazoxanide. *J. Infect. Dis.*, 2001, *184* (1), 103-106.

ROSSIGNOL J.F. Nitazoxanide in the treatment of acquired immune deficiency syndrome-related cryptosporidiosis: results of the United States compassionate use program in 365 patients. *Aliment. Pharmacol. Ther.*, 2006, *24* (5), 887-894

ROSSIGNOL J.F., KABIL S.M., YOUNIS A.M. & EL-GOHARY Y. Double-blind placebo controlled study of nitazoxanide in the treatment of cryptosporidial diarrhea in 90 immunocompetent adults and adolescents from the Nile delta of Egypt. *Clin. Gastroenterol. Hepatol.*, 2006, *4* (3), 320-324

SMITH N.H., CRON S., VALDEZ L.M., CHAPPELL C.L. & WHITE A.C. Jr. Combination drug therapy for cryptosporidiosis in AIDS. *J. Infect. Dis.*, 1998, *178* (3), 900-903

STACHULSKI A.V., BERRY N.G., LILIAN LOW A.C., MOORES S.L., ROW E., WARHURST D.C., ADAGU I.S. & ROSSIGNOL J.F. Identification

of isoflavone derivatives as effective anticryptosporidial agents *in vitro* and *in vivo*. *J. Med. Chem.*, 2006, *49* (4), 1450-1454.

THEODOS C.M., GRIFFITHS J.K., D'ONFRO J., FAIRFIELD A. & TZIPORI S. The efficacy of nitazoxanide against *C. parvum* in cell culture and in animal models. *Antimicrob. Agents Chemother.*, 1998, *42* (8), 1959-1965.

TZIPORI S. & WIDMER G. The biology of *Cryptosporidium*. *Contrib. Microbiol.*, 2000, *6*, 1-32.

UIP D.E., LIMA A.L.L., AMATO V.S., BOULOS M., NETO V.A. & BEM DAVID D. Roxithromycin treatment for diarrhoea caused by *Cryptosporidium* spp. in patients AIDS. *J. Antimicrob. Chemother.*, 1998, *41* (Suppl. B), 93-97.

VEDADI M., LEW J., ARTZ J., AMANI M., ZHAO Y., DONG A., WASNEY G.A., GAO M., HILLS T., BROKX S., QIU W., SHARMA S., DIASSITI A., ALAM Z., MELONE M., MULICHAK A., WERNIMONT A., BRAY J., LOPPNAU P., PLOTNIKOVA O., NEWBERRY K., SUNDARARAJAN E., HOUSTON S., WALKER J., TEMPEL W., BOCHKAREV A., KOZIERADZKI I., EDWARDS A., ARROWSMITH C., ROOS D., KAIN K. & HUI R. Genome-scale protein expression and structural biology of *Plasmodium falciparum* and related Apicomplexan organisms. *Mol. Biochem. Parasitol.*, 2007, *151* (1), 100-110.

WALLACE M.R., NGUYEN M.T. & NEWTON J.A.J. Use of paromomycin for the treatment of cryptosporidiosis in patients with AIDS. *Clin. Infect. Dis.*, 1993, *17* (6), 1070-1073.

WHITE A.C. JR, CHAPPELL C.L., HAYAT C.S., KIMBALL K.T., FLANIGAN T.P. & GOODGAME R.W. Paromomycin for cryptosporidiosis in AIDS: a prospective, double-blind trial. *J. Infect. Dis.*, 1994, *170* (2), 419-424.

YARLETT N., WU G., WATERS W.R., HARP J.A., WANNEMUEHLER M.J., MORADA M., ATHANASOPOULOS D., MARTINEZ M.P., UPTON S.J., MARTON L.J. & FRYDMAN B.J. *C. parvum* spermidine/spermine N1-acetyltransferase exhibits different characteristics from the host enzyme. *Mol. Biochem. Parasitol.*, 2007, *152* (2), 170-180.

YARLETT N., WATERS W.R., HARP J.A., WANNEMUEHLER M.J., MORADA M., BELLCASTRO J., UPTON S.J., MARTON L.J. & FRYDMAN B.J. Activities of DL-alpha-difluoromethylarginine and polyamine analogues against *C. parvum* infection in a T-cell receptor alpha-deficient mouse model. *Antimicrob. Agents Chemother.*, 2007, *51* (4), 1234-1239.

ZARDI E.M., PICARDI A. & AFELTRA A. Treatment of cryptosporidiosis in immunocompromised hosts. *Chemotherapy*, 2005, *51* (4), 193-196.