Cryptosporidiosis and Other Enteric Protozoan Infections in AIDS-related Diarrhea in Thailand

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Diarrhea in AIDS

- A substantial number of HIV-infected patients suffer from diarrhea caused by a wide range of opportunistic and non-opportunistic pathogens.

- It is associated with significant morbidity and mortality.

- The accurate identification of the causes of diarrhea allows appropriate treatment.
Cryptosporidiosis

- Human infection is caused by at least 8 Cryptosporidium species/genotypes.
- *C. parvum* and *C. hominis* are the most common species infect human.
- *C. meleagridis, C. felis, C. canis, C. suis, C. muris,* and *Cryptosporidium cervine* genotype also infect human.
Cryptosporidiosis

• In immunocompetent person
  – Asymptomatic carriage
  – Acute, self-limited diarrhea
  – Persistent diarrhea that may continue for weeks

• Supportive care with IV or oral rehydration fluid to correct the dehydration

• No specific Treatment is required
Cryptosporidiosis

• Clinical manifestations in patients with AIDS
  – Asymptomatic infection
  – Transient infection: CD4 > 200/ cu.mm
  – Chronic diarrhea ≥ 2 months, persistent oocyst when CD4 < 100/cu.mm.
  – Fulminant disease, > 2 L of watery diarrhea/daily if CD4 < 50/ cu.mm.

• Shorter survival than those without cryptosporidiosis.
Treatment of Cryptosporidiosis

- Antimicrobial therapy
- Immunotherapy
- Symptomatic anti-diarrheal treatment
Immune Reconstitution

• Immune reconstitution using HAART

• Useful as a treatment and secondary prophylaxis for cryptosporidiosis in HIV-infected patients.

• Protease inhibitors reduce *C. parvum* sporozoite host cell invasion and inhibit parasite development *in vitro*.

• The inhibitory effect was increased when paromomycin was combined with the PIs.
Passive Immunotherapy

• Oral bovine serum concentrate improved symptoms and reduced oocyst shedding in experimental cryptosporidial diarrhea

• Colostrum from cows hyperimmunized with *C. parvum* oocysts achieved limited success in both human and non-human hosts.
Antimicrobial Therapy

- **Macrolide**
  - Oral spiramycin
  - Azithromycin
  - Clarithromycin

- **Aminoglycoside**
  - Paramomycin

- **Nitazoxanide**
Nitazoxanide

- 2-(acetyloxy)-N-(5-nitro-2-thiazolyl) benzamide, a synthetic oral antiparasitic agent, is effective against broad range of protozoa and helminths including Cryptosporidium spp.
- It has been licensed for the treatment of giardia-associated diarrhea and cryptosporidial diarrhea in non-HIV infected children since December 2002.
- It is the first and only US FDA-approved drug for cryptosporidiosis so far.
Nitazoxanide

• In 50 HIV-negative children with cryptosporidiosis
  – Diarrhea resolved in 56% in nitazoxanide treated group compared to 23% in placebo group (P=0.037).
  – Oocyst eradication in 52% in nitazoxanide treated group compared to 14% in placebo group (P=0.007).
  – Mortality 18% at day 8th in placebo group compared to 0% in nitazoxanide treated group (P=0.04)

• No benefit was shown in 50 HIV+ve children, diarrhea resolved after a 2nd course of treatment, but few of them had parasitological clearance.

Amadi et al. Lancet 2002; 360:1375-80
Nitaxozanide

• A phase II placebo-controlled study of nitazoxanide in AIDS subjects in Mexico
  – a 14-day course of nitazoxanide at a dose of 1,000 mg bid was effective in treating cryptosporidiosis in patients with CD4 ≤ 50
  (parasitological response 67% vs 25% in placebo group)
A phase II placebo-controlled study of nitazoxanide in AIDS subjects in Mexico

- In patients with CD4 <50 cu.mm, the response rate of nitazoxanide therapy was not a significant difference from placebo.

- Higher doses and/or longer duration of therapy may be needed to obtain responses in these severely immunocompromised patients.
OBJECTIVES

• To determine the prevalence of protozoan pathogens associated with diarrhea in HIV-infected patients in Thailand.
• To compare clinical manifestation of diarrhea caused by these pathogens.
• To determine the efficacy and safety of nitazoxanide treatment for cryptosporidiosis.
Material And Methods

• A laboratory-based coprodiagnostic investigation conducted at Siriraj Hospital and Bamrasnaradura Institute, Bangkok, Thailand

• All patients with diarrhea were asked to produce a stool sample for
  • simple wet smear and formalin-ether concentrates
  • modified Ziehl-Neelsen stained smear
  • enteric bacterial and mycobacteria culture
  • Clostridium difficile toxin A assay
  • Modified trichrome blue stained smear
Material And Methods

- The identification of Cryptosporidium oocyst was confirmed by Meriflour Cryptosporidium-Giardia monoclonal direct immunofluorescence detection kit.

- The identification of Microsporididia was confirmed and speciated by thin sectioning electron microscopy.
A Randomized Placebo – Controlled Trial of Nitazoxanide for the Treatment of Cryptosporidiosis
Material and Methods

- Inclusion Criteria
  - HIV infection and CD4 counts ≤ 50 /cu.mm.
  - Age > 13 years
  - Presence of oocyst of *C. parvum*.
  - At least 3 bowel movements per day, on average, during the 5 days prior to enrollment based upon the observation of the hospital staff, and on at least 5 days a week, on average, for 21 days prior to enrollment.
  - Willingness to remain hospitalized for 5 days prior to enrollment and for the first 14 days of the study.
  - In the case of females, adequate birth control.
Material and Methods: exclusion criteria

- Inability to tolerate oral medications.
- Life expectancy < 120 days in the opinion of the investigator.
- Active CMV colitis, \textit{C. difficile} colitis, amebiasis, giardiasis, salmonellosis, shigellosis, campylobacteriosis, inflammatory bowel disease, diarrhea secondary to another documented pathogen (other than microsporidia) or symptomatic MAC disease.
- Need for continuing use of any medications with potential anticryptosporidial activity including paromomycin, azithromycin, clarithromycin, spiramycin, bovine colostrum, monoclonal anticryptosporidial antibody preparations, etc.
Material and Methods

• Study Procedure
  – Nitazoxanide/ placebo 1,000 mg bid for 4 weeks. follow by 1,500 mg bid for 4 weeks
  – Patients were examined every 2- week during the 8 week treatment and at 2, 4, 6 week after Rx
  – A stool sample was collected at each visit
  – Two stool samples were collected at 8-week of treatment and 6-week of follow up
Material and Methods

• Study Procedure
  – Treatment were discontinued in the event of parasitological “cure” and “well” clinical response on two consecutive visits.
  – Patients with persistent shedding of Cryptosporidium oocyst received nitazoxanide open label treatment of the same dosage regimen.
Outcome

• Clinical response
  – Well: < 3 bowel movements/d over the last 5 days
  – Continuing illness
  – Clinical relapse/reinfection

• Parasitological response
  – Eradication: no cryptosporidium oocyst in 2 stool samples collected at the end of treatment
  – Persistence
  – Relapse or reinfection
• Therapeutic response
  – Cure; well + eradication
  – Failure; Continuing illness or persistence
  – Relapse; a change in therapeutic response from cure at the end of treatment to failure at the follow up examination.
RESULTS

1,138 fecal samples from 909 patients were screened between November 1999- July 2004

- 683 (75.2) patients with diarrhea
  - 219 (24.1%) patients with diarrhea for <1 week.
  - 109 (12%) patients with diarrhea between one to less than 4 weeks.
  - 355 (39.1%) patients with diarrhea for ≥4 weeks.
- 54 (5.9) patients without diarrhea
- No data available in 172 (18.9%) patients.
RESULTS

- Male : female = 2:1
- Median age was 34 (range 15 to 67) years.
- CD4 was measured in 334 patients
  - Median CD$_4$ count was 25 cell/cu.mm.
    - (range 1 - 714)
  - 65.2% of them had CD4 count < 50 cell/cu.mm.
- Overall protozoan or helminthic infection were found in 432 (47.5%) patients.
- Dual pathogens were found in 64 (7%) patients
Protozoan pathogens identified in the study group (909 patients)

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cryptosporidium</em> oocyst</td>
<td>193</td>
<td>(21.5%)</td>
</tr>
<tr>
<td><em>Microsporidian</em> spore</td>
<td>101</td>
<td>(11.1%)</td>
</tr>
<tr>
<td><em>Isospora</em> oocyst</td>
<td>49</td>
<td>(5.4%)</td>
</tr>
<tr>
<td><em>Giardia lamblia</em></td>
<td>30</td>
<td>(3.3%)</td>
</tr>
<tr>
<td><em>Entamoeba histolytica</em></td>
<td>4</td>
<td>(0.4%)</td>
</tr>
<tr>
<td><em>Cyclospora</em></td>
<td>5</td>
<td>(0.5%)</td>
</tr>
<tr>
<td><em>Strongyloides stercoralis</em></td>
<td>40</td>
<td>(4.4%)</td>
</tr>
<tr>
<td>Bacterial Pathogens</td>
<td>Count</td>
<td>Percentage</td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>-------</td>
<td>------------</td>
</tr>
<tr>
<td><em>Clostridium difficile</em></td>
<td>16</td>
<td>(15.6%)</td>
</tr>
<tr>
<td>Mycobacteria</td>
<td>18</td>
<td>(12.9%)</td>
</tr>
<tr>
<td><em>Salmonella spp.</em></td>
<td>11</td>
<td>(4.4%)</td>
</tr>
<tr>
<td><em>Campylobacter spp.</em></td>
<td>18</td>
<td>(7.1%)</td>
</tr>
<tr>
<td>Shigella spp.</td>
<td>1</td>
<td>(0.4%)</td>
</tr>
<tr>
<td>Total</td>
<td>64</td>
<td>(22.2%)</td>
</tr>
<tr>
<td></td>
<td>Cryptosporidium-infected group</td>
<td>Microsporidium-infected group</td>
</tr>
<tr>
<td>----------------------</td>
<td>-------------------------------</td>
<td>------------------------------</td>
</tr>
<tr>
<td>Total number</td>
<td>160</td>
<td>76</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>85 (53.1)</td>
<td>46 (60.5)</td>
</tr>
<tr>
<td>Median (range) age, yrs</td>
<td>34 (21-58)</td>
<td>32 (20-48)</td>
</tr>
<tr>
<td>Median (range) CD4, cell/ml</td>
<td>11 (1-554)</td>
<td>20 (4-707)</td>
</tr>
<tr>
<td>- CD4&lt;50 cell/ml, n/total (%)</td>
<td>73/83 (88)</td>
<td>26/40 (65)</td>
</tr>
<tr>
<td>Diarrhea, n/total (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- No</td>
<td>6/136 (4.4)</td>
<td>4/68 (5.9)</td>
</tr>
<tr>
<td>- less than 7 days</td>
<td>30/136 (22.1)</td>
<td>11/68 (16.2)</td>
</tr>
<tr>
<td>- between 7-30 days</td>
<td>24/136 (17.6)</td>
<td>7/68 (10.3)</td>
</tr>
<tr>
<td>- more than 30 days</td>
<td>76/136 (55.9)</td>
<td>46/68 (67.6)</td>
</tr>
<tr>
<td>Weight lost, n/total (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outcome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Dead, n/total (%)</td>
<td>68/160 (42.5)</td>
<td>9/76 (11.8)</td>
</tr>
</tbody>
</table>
Genotyping: 34 samples

- C. hominis (C. parvum human genotype, 50%)
  - C. meleagridis (20%)
  - C. parvum (15%)
  - C. felis (4%)
  - C. canis (4%)

(using RFLP and sequencing of 18sRNA gene)

# Human Cryptosporidiosis

<table>
<thead>
<tr>
<th>Location</th>
<th>Type of patients</th>
<th>Total no. of patients</th>
<th>No. of patients infected with:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>C. hominis</td>
</tr>
<tr>
<td>Portugal</td>
<td>HIV+</td>
<td>29</td>
<td>7</td>
</tr>
<tr>
<td>Switzerland</td>
<td>HIV+</td>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td>France</td>
<td>HIV+</td>
<td>46</td>
<td>14</td>
</tr>
<tr>
<td>Thailand</td>
<td>HIV+</td>
<td>29</td>
<td>24</td>
</tr>
<tr>
<td>Thailand</td>
<td>HIV+</td>
<td>34</td>
<td>17</td>
</tr>
<tr>
<td>Atlanta</td>
<td>HIV+</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>New Orleans</td>
<td>HIV+</td>
<td>29</td>
<td>18</td>
</tr>
<tr>
<td>Peru</td>
<td>HIV+</td>
<td>118</td>
<td>76</td>
</tr>
<tr>
<td>Peru</td>
<td>Children</td>
<td>83</td>
<td>65</td>
</tr>
<tr>
<td>Kenya</td>
<td>All</td>
<td>33</td>
<td>23</td>
</tr>
<tr>
<td>Japan</td>
<td>All</td>
<td>22</td>
<td>16</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>All</td>
<td>1,680–2,057</td>
<td>815</td>
</tr>
</tbody>
</table>

* Only data from studies using PCR that amplifies all five Cryptosporidium spp. are quoted.

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A Randomized Placebo – Controlled Trial of Nitazoxanide for the Treatment of Cryptosporidiosis: Nov 1999- June 2004

RESULTS
- 50 patients were studied
  - 37 patients were enrolled by Jan 2002
  - 11/37 patients died from advance AIDS.
- Overall 19 (32%) patients died,
- 6 (12%) patients lost to follow up.
Outcome : Up to Jan 2002

• Dead
  – Leukemia, lymphoma 2
  – severe wasting 7
  – MAC septicemia 1
  – Cryptococcal septicemia 1

• On ARV ( DDI+ D4Tor D4T+3TC+ nevirapine)
  – improved 8
  – failure 2
  – initial therapy 2
Cryptosporidiosis
Patient Disposition Flow Chart

50 Enrolled

25 Nitazoxanide
- 10 Withdrawn
  -10 due to adverse events
15 Completed Study

25 Placebo
- 12 Withdrawn
  -11 due to adverse events
  -1 lost on follow-up
13 Completed Study
## Demographic and Disease–Related Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Nitazoxanide</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male: Female</strong></td>
<td>20:21</td>
<td>9:13</td>
<td>11:8</td>
</tr>
<tr>
<td><strong>Age, yr</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>34.3 (7)</td>
<td>33.8 (6)</td>
<td>34.4 (9)</td>
</tr>
<tr>
<td>Range</td>
<td>21-62</td>
<td>23-46</td>
<td>21-62</td>
</tr>
<tr>
<td><strong>CD4 count, cu.mm.</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>9.4 (6.8)</td>
<td>10.9 (7)</td>
<td>7.7 (6)</td>
</tr>
<tr>
<td>Range</td>
<td>2-31</td>
<td>3-31</td>
<td>2-24</td>
</tr>
<tr>
<td><strong>Duration of Diarrhea, d</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>90 (21-720)</td>
<td>90 (21-720)</td>
<td>90 (21-450)</td>
</tr>
<tr>
<td><strong>Oocyst count, n</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Many</td>
<td>27</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>- Moderate</td>
<td>6</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>- Few</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>- Rare</td>
<td>5</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Nitazoxanide</td>
<td>Placebo</td>
<td>P-value</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>--------------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td><strong>n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Clinical response</strong></td>
<td></td>
<td></td>
<td>0.049</td>
</tr>
<tr>
<td>Well</td>
<td>7 (32)</td>
<td>1 (5)</td>
<td></td>
</tr>
<tr>
<td>Continuing illness</td>
<td>15 (68)</td>
<td>18 (95)</td>
<td></td>
</tr>
<tr>
<td><strong>Parasitological response</strong></td>
<td></td>
<td></td>
<td>0.49</td>
</tr>
<tr>
<td>Eradication</td>
<td>2 (9)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Persistence</td>
<td>20 (91)</td>
<td>19 (100)</td>
<td></td>
</tr>
<tr>
<td><strong>Therapeutic response</strong></td>
<td></td>
<td></td>
<td>0.49</td>
</tr>
<tr>
<td>Cure</td>
<td>2 (9)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Failure</td>
<td>20 (91)</td>
<td>19 (100)</td>
<td></td>
</tr>
</tbody>
</table>
48/50 patients reported AE
- 86 AE in nitaxozanide group and 78 AR in placebo group
- Most AEs were not related to the drug except mild or transient yellowish sclera in 6/25 nitaxozanide group

6 deaths in nitaxozanide and 5 deaths in placebo group
14 patients were entered the study
- 10/14 showed “well” clinical responses
- 1/14 absent of cryptosporidium oocyst but relapse at the end of 8 week treatment
Incidence/1,000 person-years

HAART


PCP
Mycobacterium avium complex
Esophageal candidiasis
CMV retinitis
CMV disease
KS
Extrapulmonary cryptococcosis
Toxoplasmosis

Morris et al.
EID 2004
Opportunistic protozoa in stool samples from HIV-infected patients

- 22 HIV-infected patients with chronic diarrhea (>3 weeks)
  - Microsporidia 6 (27.3%)
  - Cryptosporidia 2 (9%)
  - Isospora belli 1 (4.5%)
  - Giardia intestinalis 2 (9.1%)
  - Candida spp. 7 (31.8%)
  - Strongyloides stercolaris 3 (13.6%)
  - Opisthorchis viverrini ova 1 (4.6%)

Punpoowong et al. 1998
%Intestinal Parasite in Non-HIV and HIV Infected Patients
Intestinal Parasitic Infection: Siriraj Hospital (1999-2005)

- The overall prevalence was 18.9%
- Infection rate was 44.9% in HIV & 15.6% in non-HIV patient
- Helminthic infection was found in 8.9% of both groups.
- The Prevalence of protozoan infection was 29.6% in HIV group versus 3.9% in non-HIV group
- The prevalence of *Cryptosporidium spp.* was 20.7%, and
- The prevalence of microsporidial infection was 15.5% in HIV patients
Prevalence of *Cryptosporidium* in Thailand: 2007

- 46 HIV patients from Prabat Numpu Temple, Lopburi, Thailand
- The prevalence was 28.3% (13/46)
- 5/13 (15.1%) of patients with diarrhea and 8/33 (24.2%) of patients without diarrhea
- Four isolates were confirmed to be *C. parvum* by genotyping

Nuchjangreer C. et al Parasitol Res. 2008
Conclusion

- *Cryptosporidium spp.* remains a significant intestinal protozoan in HIV–infected patients in Thailand.
- Immune reconstitution is the key to eradication and prevention of cryptosporidiosis among these HIV-infected patients.
Nitazoxanide is effective against cryptosporidiosis, but only in patients with CD4 >50.

The efficacy of nitazoxanide was not significant different from placebo in this small study of patients with CD4 <50.

High dose, and 8 week duration of treatment was well tolerated.
Conclusion

• In patients unable to take ARV, cryptosporidium diarrhea remains a challenging disease.
• More studies such as Cryptosporidium genome will assist to the discovering of new gene, biochemical pathways and protective antigens that can be targeted to develop novel therapies for cryptosporidiosis.
• Prof. J.F. Rosignol, Romark Institute for Clinical Research, Florida USA
• Dr. Methee Chayakulkeeree
• Surapee Triengrim
• Nurses and laboratory technicians at Siriraj Hospital and Bamrasnaradura Institute, Ministry of Public Health