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Intestinal Protozoa in HIV-Infected Patients: Effect of Rifaximin in *Cryptosporidium parvum* and *Blastocystis hominis* Infections

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Summary

In HIV-1 infected patients severe enteritis and chronic diarrhea are often documented as a consequence of multiple opportunistic infections. We analyzed 48 HIV-1 positive patients for the presence of intestinal pathogenic protozoa. Patients with $CD4 \geq 200/mm^3$ showed a higher prevalence of a single pathogenic protozoa than patients with $CD4 \leq 200/mm^3$, who showed the presence of multiple protozoal infections. Patients who proved positive for only a single protozoa, *Cryptosporidium* or *Blastocystis*, were also positive, by stool culture, for the presence of *Proteus mirabilis* (3 samples), *Citrobacter freundii* (3 samples), *Escherichia coli* (one sample) or *Enterobacter cloacae* (one sample). Treatment with rifaximin (600 mg, 3 times a day, for 14 days) was efficacious in resolving the clinical symptoms and clearing protozoan infections in HIV-1 infected patients with $CD4 \geq 200/mm^3$, who presented enteric and systemic symptoms due to *Cryptosporidium* or *Blastocystis* associated with enteropathogenic bacteria.

Key words: AIDS, chronic diarrhea, *Cryptosporidium*, *Blastocystis*, rifaximin.

INTRODUCTION

Opportunistic protozoa are frequently pathogenic in HIV-infected patients. In these subjects intestinal protozoa are a common cause of severe enteritis and chronic diarrhea. The ill-

ness can be complicated by dehydration and malabsorption and can be life threatening.

Cryptosporidium parvum is among the more common opportunistic intestinal pathogens in HIV-infected patients with an incidence between 5%¹ and 38%². Intestinal

cryptosporidiosis is characterized by the presence of profuse and watery diarrhea, dehydration, rapid weight loss, and sometimes nausea, vomiting, abdominal pain and fever. Once the infection is established in patients with HIV infection, the great majority of patients have lifelong infection that is refractory to treatment. Numerous drugs have been analyzed in humans infected by *Cryptosporidium*, including paromomycin, spiramycin, azithromycin, clarithromycin, octreotide, hyperimmune bovine colostrum, bovine transfer factor and many others. Because only limited numbers of trials have been conducted with potential therapeutic agents, the majority of information to date is preliminary in nature ³.

Blastocystis hominis has for several decades been considered a harmless yeast commensal of the intestine but is nowadays classified as a pathogenic protozoa. In immunocompetent hosts *B. hominis* may be the cause of an acute self limited diarrhea while in immunocompromised hosts it can cause prolonged or recurrent diarrhea which can be associated with abdominal pain, flatus, anorexia, fever and sometimes eosinophilia. The success of treatment with standard antiprotozoan agents has been shown to be variable ^{4,5}.

In HIV-infected patients diagnostic and therapeutic approaches to enteric infections have mainly been based on a single etiological agent although some of these patients may harbor multiple pathogens; moreover combination antiretroviral therapy that includes a protease inhibitor can restore immunity to *C. parvum* in HIV-infected individuals and result in sustained clinical and microbiological responses ⁶.

All these data prompted us to determine the prevalence of *C. parvum* and *B. hominis* in HIV-infected patients, treated with protease inhibitors, and evaluate the possibility of coinfection with other bacterial agents. Moreover, since rifaximin, an antimicrobial compound derived from rifamycin SV ⁷, has been shown to have an elective role in the treatment of intestinal infections due to its broad antimicrobial spectrum, minimal intestinal absorption and no significant side effects, in our study we determined the efficacy of rifaximin on the treatment of *C. parvum* and *B. hominis* intestinal infections. The aim of this study was to evaluate the efficacy and tolerability of rifaximin in patients with stool cultures positive for protozoan pathogens.

PATIENTS AND METHODS

Study population

From March 1996 to March 1998, 48 HIV-seropositive patients, 26 females and 22 males, with an average age of 28 years (range 12-54), with enteric symptoms (mainly diarrhea) and/or eosinophilia, were enrolled in the study.

HIV seropositivity was determined by the presence of anti HIV-1 antibodies detected by ELISA and immunoblotting. CD4+ lymphocyte count was established by cytofluorimetric assay and expressed as absolute number/mm³. At the initial visit a standardized interview was administered and a physical examination performed. All patients gave informed consent before evaluation.

Out of the 48 patients, 14 had CD4 values <200/mm³ and all of them received antiretroviral therapy including at least one protease inhibitor; in detail 8 patients received indinavir (IDV) plus two reverse transcriptase inhibitors (RTI), 5 patients received ritonavir (RTV) plus two RTI and one patient received saquinavir (SQV) plus two RTI. All these 14 patients had received the therapy for no less than 14 days and no longer than 3 months.

Out of the 48 enrolled patients, 34 showed CD4 values >200/mm³ and 9 did not receive any therapy while 25 received antiretroviral therapy; in detail 10 patients received IDV plus two RTI, 7 patients received RTV plus two RTI, 6 patients received SQV plus two RTI and 2 patients 2 RTI only. These 25 patients received the therapy for no less than one month and no longer than 15 months.

Fecal samples

Three fecal samples for each patient were collected every other day and were transported within 30 min to the laboratory. The samples were processed for parasitological and bacteriological analysis. Samples were examined microscopically for ova, cysts and parasites with iodine stain after concentration. The examination was complemented by the modified Ziehl-Neelsen technique in search of *C. parvum* ⁸. In brief, smears fixed in methanol for 3 min were stained with carbolfuchsin for 5 min and then decolorization in 3% hydrochloric acid in 93% ethanol was performed. The smears were rinsed in tap water and then counterstained in

0.25% malachite green for 30 sec, rinsed again and air dried.

To search for intestinal pathogenic bacteria, stool specimens were inoculated into blood agar and selective/differential agar plates for intestinal bacteria.

Therapy

Patients positive for pathogens which were identified by the techniques described above were treated with rifaximin (Normix[®] tablets 200 mg), 600 mg three times per day for 14 days. During and after rifaximin therapy, each patient was examined by the investigators who obtained a detailed gastrointestinal history to assess the patient’s response to therapy. A daily diary of potential adverse reactions to the drug was kept. The following parameters were evaluated: bowel motions, stool characteristics, abdominal pain, fever. Microbiological responses were evaluated for clearing of the infectious agents.

RESULTS

Out of the 48 HIV-infected patients studied for parasitological examination, 15 (31%) were positive for one or more protozoa and 33 (69%) proved negative, showing no potentially infectious pathogens in the stools.

The results of the parasitological examination of the 15 positive patients along with the patients’ CD4 values are shown in *Table 1*. In all 6 patients with CD4 values >200/mm³ only one pathogenic protozoa species was identified for each patient. In the 9 patients with CD4 values <200/mm³, 5 patients had a single pathogenic protozoa in their stools while 4 patients had two different pathogenic protozoa concomitantly. Three subjects had *C. parvum* only, one had *Giardia intestinalis*, one had *Isospora belli*, while 3 out of the 4 patients

with multiple protozoan infections concomitantly had *C. parvum* and *Giardia* and one *C. parvum* and *B. hominis*.

The bacteriological stool culture in the 8 patients positive for *C. parvum* (6 patients) or *B. hominis* (2 patients) as a single pathogenic protozoa showed the copresence of different pathogenic and non-pathogenic bacteria: *Proteus mirabilis* (3 samples), *Citrobacter freundii* (3 samples), *Escherichia coli* (one sample), *Enterobacter cloacae* (one sample). In order to evaluate the efficacy of rifaximin in the *C. parvum* and *B. hominis* infections with the copresence of bacterial infections, the 3 patients with CD4 values >200/mm³ who were positive both for *C. parvum* and enteropathogenic bacteria, and the 2 patients with CD4 values >200/mm³ positive both for *B. hominis* and enteropathogenic bacteria were treated with rifaximin.

The main clinical features evaluated during the course of rifaximin therapy are summarized in *Table 2*.

As regards bowel motions, all treated patients but one showed symptomatic improvement on the fourth day. At the beginning of the therapy, all 5 treated patients had loose or watery stools which became formed after the fourth day of therapy. Medium abdominal pain was present at the beginning of therapy in 3 patients and after 4 days disappeared in 2 and in one became mild. The 2 patients with fever >37°C at the beginning of the therapy had a temperature of ≤37°C on the second day of therapy.

After 4 days of therapy all treated patients experienced a complete remission of all symptoms (fever, abdominal pain and diarrhea). After completing the course of rifaximin therapy, which occurred without any side effects, the 5 patients were examined for the presence of stool parasites, three times at 2-day intervals. All 5 patients proved negative in all the samples tested.

TABLE 1 - Intestinal protozoa in HIV patients.

	<i>C. parvum</i> alone	<i>C. parvum</i> + <i>G. intestinalis</i>	<i>G. intestinalis</i>	<i>C. parvum</i> + <i>B. hominis</i>	<i>B. hominis</i>	<i>I. belli</i>
CD4 >200 (N. PTS.)	3	0	1	0	2	0
CD4 <200 (N. PTS.)	3	3	1	1	0	1

TABLE 2 - Clinical monitoring of *C. parvum* and *B. hominis* patients.

Days on treatment	N. bowel movements				Stool characteristics				Abdominal pain				Fever (°C)							
	base-line	1	2	3	4	base-line	1	2	3	4	base-line	1	2	3	4					
c1*	3	3	2	1	1	L**	L	L	F	F	ME§	ME	ME	A	A	36.5	36.5	36.5	36.5	36.5
c2	3	3	2	2	2	W	W	W	W	F	ME	MI	MI	MI	MI	37.5	37.5	36.5	36.5	36.5
c3	3	2	2	1	1	L	L	F	F	F	ME	ME	ME	ME	A	38.5	38.5	37	36.5	36.5
b1	4	4	4	4	2	W	W	L	L	F	A	A	A	A	A	36.5	36.5	36.5	36.5	36.5
b2	3	3	3	2	1	L	L	L	F	F	A	A	A	A	A	36.5	36.5	36.5	36.5	36.5

*C=Pt With *C. parvum* **L=Loose §ME=Medium B=Pt With *B. hominis* F=Formed A=Absent W=Watery MI=Mild

DISCUSSION

Several opportunistic infections have been documented in patients with HIV-1 infections, of which those of the gastrointestinal tract are prominent. This study documents the prevalence of intestinal protozoan infections in a study group of 48 HIV infected subjects on antiretroviral treatment with protease inhibitors showing enteric and systemic symptoms. The most frequently identified intestinal protozoa was *C. parvum*, which was present either alone or with other intestinal protozoa in 10 patients. The next most frequently identified intestinal protozoa was *G. intestinalis* (5 patients) followed by *B. hominis* (3 patients) and *I. belli* (one patient). Multiple protozoan infections were noted in 4 patients who had CD4 values <200/mm³. This demonstrates that multiple opportunistic infections are more frequent in subjects with a higher grade of immunodeficiency.

C. parvum, either alone or with other intestinal pathogens, was found in 10 cases (20.8%) showing a decreased incidence with respect to the values of 33% found in another study, also performed in Italy in 1993⁹. This decreased value can be due to the fact that our patients were treated with the new combination antiretroviral therapy including protease inhibitors which has been shown to restore immunity to *C. parvum*⁶. The decreased incidence of pathogenic protozoa such as *C. parvum* has been balanced by the increase in low pathogenic opportunistic protozoa such as

B. hominis^{10,11}. In our study the presence of *Blastocystis*, either alone or with other intestinal protozoa, was documented in 6.2% patients.

Since clinical intestinal manifestations in HIV subjects can also be due to the concomitant presence of protozoa and bacteria, in our study we evaluated the presence of enteropathogenic bacteria in the 8 patients who had *C. parvum* or *B. hominis* as a single protozoan agent and all proved positive for the presence of bacteria. Among these 8 patients, 5 had CD4 values >200/mm³ and thus were chosen to be treated with rifaximin. We excluded the 3 subjects with CD4 values <200/mm³ from the rifaximin treatment, since in these highly immunocompromised patients specific antiprotozoan therapy has been shown to have limited validity¹². All patients who were positive for *B. hominis* and *C. parvum* and also showed the presence of another microorganism, had a complete resolution of symptoms after 4 days of therapy when treated with rifaximin, with clearance of *C. parvum* and *B. hominis*. Therapy with oral rifaximin was well tolerated in all patients and no specific complaints were related to the therapy. Since the presence of *C. parvum* and *B. hominis* had been accompanied by positivity for enterobacteria in our rifaximin treated patients, we believe that by acting as a regulator of intestinal bacterial population rifaximin can alter the balance between infecting intestinal protozoa and other coinfecting intestinal bacteria.

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