Clin Investig (1993) 71:42–45

Original Article

Clinical Investigator

A preparation from bovine colostrum in the treatment of HIV-positive patients with chronic diarrhea

A. Plettenberg[1], A. Stoehr[1], H.-J. Stellbrink[2], H. Albrecht[2], and W. Meigel[1]
[1] Allgemeines Krankenhaus St. Georg, HIV-Ambulanz, Hamburg
[2] Universitätsklinik Eppendorf, Medizinische Poliklinik, Hamburg

Summary. In a prospective, open, uncontrolled study 25 patients infected with the human immunodeficiency virus with chronic refractory diarrhea and either confirmed cryptosporidiosis ($n=7$) or absence of demonstrable pathogenic organisms ($n=18$) were treated with a daily oral dose of 10 g of an immunoglobulin preparation from bovine colostrum over a period of 10 days. Among the 7 patients with cryptosporidiosis, this treatment led to complete remission in 3 and partial remission in 2. Among the 18 patients with diarrhea and negative stool culture, complete remission of diarrhea was obtained in 7 and partial remission in 4. In the remaining 2 patients with cryptosporidiosis and the 7 patients with diarrhea but no demonstrable pathogens treatment produced no significant improvement of the diarrhea. Subsequent doubling of the Lactobin dose (2×10 g daily) in 8 of the nonresponders led to complete remission in one case and at least partial remission in a further 4 patients. Treatment of refractory diarrhea with 10 g immunoglobulins from bovine colostrum per day constitutes an important therapeutic approach and led to complete (40%) or partial (24%) remission of diarrhea in 64% of the patients described here.

Key words: AIDS – Diarrhea – Bovine colostrum – Cryptosporidiosis

Chronic diarrhea not significantly affected by therapeutic measures can be life threatening in patients positive for human immunodeficiency virus (HIV) with advanced immune deficiency [5, 16]. Common diagnostic examinations in such persistent diarrhea include repeated stool cultures and endoscopy of the lower intestinal tract with biopsies for cultural and histological examination [6, 14]. In a proportion of patients with diarrhea these diagnostic procedures lead to detection of the responsible organisms and thus permit specific treatment. Colitis due to cytomegaly virus and bacterial enteritis can be successfully treated in this way [2, 17]. If these diagnostic measures demonstrate no pathogen, nonspecific antibiotic treatment is frequently attempted. In the case of persistent clinical symptoms, use of the various peristalsis inhibitors is often the last remaining therapeutic option [5, 6].

In everyday practice two groups of patients with treatment-resistant diarrhea are of special importance. These are (a) those in whom pathogens are not demonstrated despite marked clinical symptoms and (b) those with confirmed cryptosporidiosis. For the latter there is as yet no acknowledged therapeutic concept [14]. We report here on a series of HIV-positive patients complaining of chronic diarrhea, without demonstrable pathogens or with confirmed cryptosporidiosis, who were treated with a preparation from bovine colostrum, Lactobin (Biotest Pharma, Dreieich).

Patients and methods

In a prospective, open, uncontrolled study 25 HIV-positive patients suffering from chronic diarrhea, with negative stool cultures or with cryptosporidiosis, were treated with Lactobin in the outpatient departments of the St. Georg General Hospital and the Universitätsklinik in Eppendorf. All patients had been suffering from at least three attacks of diarrhea per day for 1 month or more. In every case at least one stool specimen was cultured in different medias and investigated for *Salmonella*, *Shigella*, *Yersinia*, *Campylobacter*, *Chlamydia*, and *Cryptosporidium*. In addition, in all patients colonoscopy with biopsies of different sites with subsequent histological, bacteriological, and virological investigations was performed. The histological slices were stained according to hematoxylin-eosin, periodic acid – Schiff, Giemsa, and Ziehl-Neelsen methods. After mechanical pretreatment the biopsies for bacterial investigations were cultured like the stool specimens; the virological diagnosis was performed in human fibroblasts and green monkey kidney cell culture.

Abbreviations: HIV = human immunodeficiency virus; AIDS = acquired immunodeficiency syndrome

In 18 patients the results of this diagnostic procedure were negative. The macroscopy aspect of the coloscopy wass regular in 12 patients; in 6 cases there were patches of erythema. In the remaining 7 patients cryptosporidiosis was diagnosed. In all 7 cases the cryptosporidiosis was confirmed by biopsies; in addition, in stool specimens of 4 of these patients *Cryptosporidium* was found. In 3 of the 7 patients the macroscopy aspect of the mucous membrane was normal; in 4 patients focal or continuous erythema was seen. During Lactobin treatment and afterwards there was no endoscopy or diagnosis of stool prescribed. In every patient treatment with 500 mg ciprofloxacin twice daily for at least 5 days was attempted without success before commencing Lactobin therapy.

The ages of the 25 men admitted to the study ranged between 26 and 58 years (average 40 years). The complete clinical picture of acquired immunodeficiency syndrome (AIDS) was seen in 16 patients, while the other 9 belonged to Center for Disease Control stage IV C2. In 22 patients the number of CD4 lymphocytes was fewer than 100 cells/nl and in the other 3 cases 110, 140, and 300 cells/nl. In the history of 4 patients there occurred infections with opportunistic pathogens that also can cause diarrhea. These were cytomegalovirus retinitis in 2, cytomeglovirus colitis in 1, and *Mycobacterium avium* infection of the respiratory tract also in 1 patient.

After admission to the study each of the 25 patients received 10 g Lactobin, which was dissolved in warm water and taken on an empty stomach on ten consecutive mornings. A proportion of patients in whom 10 g Lactobin daily produced no complete or partial remission of diarrhea was subsequently treated with 10 g Lactobin twice daily (morning and evening) for a further 10 days. During the Lactobin therapy the patients received no antibiotics or peristalsis inhibitors. The preparation Lactobin (Biotest Pharma, Dreieich) is obtained from the colostrum of cows. A high titer of antibodies against a variety of organisms pathogenic to humans and animals was demonstrated in the preparation [19]. The solution, ready for drinking (100 ml), contains about 4.5 g IgG, 0.5 g IgM, and 1.5 g IgA. The prepared solution has a pH of around 4.5.

Therapeutic results were assessed after 5, 10, and (where applicable) 20 days of Lactobin treatment. Complete remission was defined as total remission of diarrhea at the time of assessment, while a reduction in the frequency of diarrhea by 50% or more was interpreted as partial remission. All patients who continued to experience more than half the original stool frequency were considered nonresponders. In 18 of the 25 patients no pathogens were detected despite the diagnostic procedure described above. In the other 7 cases cryptosporidiosis was confirmed. In the 18 patients with diarrhea in the absence of demonstrable pathogens the mean CD4 lymphocyte count was 76 cells/nl, and 38 cells/nl in patients with cryptosporidiosis. The differences were evaluated using the Wilcoxon (Pratt) test (two-tailed).

Results

In the 18 subjects with diarrhea and negative stool culture, 10 days of treatment with 10 g Lactobin daily per patient produced complete remission in 7 (39%) and partial remission in 4 (22%) cases. In the remaining 7 cases (39%) diarrhea was essentially unchanged after 10 days of Lactobin treatment or had diminished by less than 50% (Table 1). The frequency of diarrhea in the 18 patients fell from 5.6 (median 5.0, minimum 4.0; maximum 11.0) to 3.1 (median 2.5, minimum 0.0, maximum 10.0; Table 2). According to the Wilcoxon (Pratt) test this difference was statistically significant ($P = 0.0003$).

In the 7 patients with cryptosporidiosis, 10 days of treatment led to complete remission in 3 (43%) and partial remission in 2 (28.5%). No improvement was noted in 2 patients (28.5%; Table 3). The frequency of diarrhea in these 7 patients fell from 9.4 (median 7.0, minimum 4.0, maximum 25.0) to 3.7 (median 3.0, minimum 0.0, maximum 11.0; Table 2). Again, the Wilcoxon (Pratt) test indicated the difference to be statistically significant ($P = 0.0031$).

Table 1. Results of Lactobin treatment in patients with diarrhea and negative stool culture ($n = 18$)

Lactobin dosage	Complete remission	Partial remission	Non-responder
10 g over 5 days	5/18 (28%)	3/18 (17%)	10/18 (55%)
10 g over 10 days	7/18 (39%)	4/18 (22%)	7/18 (39%)

Table 2. Change in stool frequency after Lactobin treatment

	Patients with negative stool culture ($n = 18$)	Patients with cryptosporidiosis ($n = 7$)
At start of treatment (untreated)	5.6 ± 1.61	9.4 ± 7.28
After 5 days of treatment with 10 g Lactobin/day	3.4 ± 2.62	3.9 ± 2.91
After 10 days of treatment with 10 g Lactobin/day	3.1 ± 2.73	3.7 ± 3.99

Table 3. Results of Lactobin treatment in patients with confirmed cryptosporidiosis ($n=7$)

Lactobin dosage	Complete remission	Partial remission	Nonresponders
10 g over 5 days	3/7 (43%)	1/7 (14%)	3/7 (43%)
10 g over 10 days	3/7 (43%)	2/7 (28.5%)	2/7 (28.5%)

After the first 10 days had elapsed, 8 of the 9 nonresponders were treated with 20 g Lactobin. Of these, 2 patients had cryptosporidiosis while the other 7 patients had diarrhea without demonstrable pathogens.

The doubled Lactobin dose led to complete remission of diarrhea in one case (previously diarrhea without demonstrable pathogens) and to partial remission in another 4 cases (3 × diarrhea without demonstrable pathogens, 1 × cryptosporidiosis).

In patients with complete remission repeated diarrhea occurred in only 1 of 7 patients with initial negative stool cultures and in none of the 3 patients with cryptosporidiosis during a follow-up period of 4 weeks after withdrawal of Lactobin therapy.

Out of the total of 25 patients 6 reported minor intestinal symptoms such as nausea and flatulence after taking Lactobin, but in no case did this lead to premature discontinuation of treatment. No other side effects occurred.

Discussion

It is known from various in vivo and in vitro studies that bovine as well as human colostrum possesses broad-spectrum antimicrobial action [3, 4, 8, 9, 18, 22]. It appears to be of considerable importance that antibodies of bovine colostrum suffer hardly any inactivation by proteolytic processes during gastrointestinal passage [11]. In some of the reported treatment studies cows were initially vaccinated with various organisms to achieve a particularly high colostral concentration of certain antibodies [7, 12, 13, 20, 21]. Davidson et al. [1] reported on passive immunization with bovine hyperimmune colostrum which significantly reduced the probability of rotavirus infections in infants. Several teams have shown that diarrhea due to *Escherichia coli* could be treated very effectively with colostrum obtained from cows vaccinated against *E. coli* [7, 12]. Tzipori et al. reported the case of a 3-year-old boy with hypogammaglobulinemia in whom cryptosporidiosis associated with severe diarrhea was successfully treated with bovine hyperimmune colostrum [20]. There are also first reports on the successful use of bovine hyperimmune colostrum for diarrhea due to cryptosporidiosis in patients with AIDS [13, 21]. Ungar et al. [21] reported on one patient with AIDS and *Cryptosporidium*-related diarrhea for more than 3 months. After receiving bovine colostrum hyperimmune to *Cryptosporidium* the diarrhea disappeared. Nord et al. [13] treated 3 patients with AIDS and cryptosporidial diarrhea with bovine hyperimmune colostrum. In one patient there occurred a reduction in diarrhea and in the concentration of oocysts excreted. In a second patient a modest decrease in oocysts excreted was observed.

The exact mechanisms of action of colostrum have not yet been fully elucidated [21]. It may be assumed that the immunoglobulins present in the colostrum bind to potential pathogens or toxins in the intestinal tract and thereby facilitate their excretion. The content of IgA, which is known to have a particular protective action on the mucosa, may be of special importance. It has also been suggested that IgG1, which represents some 70% of the immunoglobulins of Lactobin [15], has an important function in the mediation of local intestinal immunity [10].

In the study presented here the preparation used was derived from the colostrum of cows, which had not previously undergone specific immunization. However, as cows are natural hosts of *Cryptosporidium* pathogenic to humans, it is not surprising that specific antibodies against these organisms are found in the preparation [19].

The reported results have shown that in patients with chronic refractory diarrhea and negative stool culture daily treatment with 10 g Lactobin was able to produce complete remission in 7 out of 18 cases (39%) and partial remission in 4 (22%). In the remaining 7 patients (39%) the treatment yielded no improvement. It should be borne in mind, however, that the etiology of diarrhea is unclear in this patient group. It is quite conceivable that some cases of HIV-associated diarrhea with negative stool culture are not due exclusively to infection of the intestinal tract. However, diarrhea in patients in whom oocytes of *Cryptosporidium* are demonstrated in the feces is very likely to be caused by these organisms. In existing cryptosporidiosis complete remission with Lactobin therapy was obtained in 3 out of 7 and partial remission in 2 cases.

It is striking that in 3 of the 4 patients with cryptopsoridia, in whom complete remission could not be achieved, daily stool frequency was 10 or above at the start of treatment. Possibly in these

cases the Lactobin was present in the intestine for too short a time to cure the infection.

After the first 10 days, the Lactobin dosage was doubled for a further 10 days in 8 of the nonresponders. This led to complete remission of diarrhea in one patient and to partial remission in another 4 patients. This raises the question of whether a higher dose or perhaps longer treatment might produce a higher response rate.

As most forms of treatment for cryptosporidiosis have so far proven unsuccessful, treatment with a preparation derived from bovine colostrum constitutes a very important approach for this indication. This applies equally to patients with negative stool cultures and to those who require urgent treatment until stool cultures are available, and specific antibiotic therapy can be instituted.

Caused by the variability of the clinical course of chronic diarrhea, such an open study without an untreated control group has a limited significance. To verify the efficiency of Lactobin treatment a placebo-controlled multicenter study is now being conducted. Further investigations about the mechanism of action, optimal dosage, and kinetics are needed.

References

1. Davidson GP, Daniels E, Nunan H, Moore AG, Whuyte PBD, Franklin K, McCloud PI, Moore DJ (1989) Passive immunisation of children with bovine colostrum containing antibodies to human rotavirus. Lancet 2:709–714
2. Dieterich DT, Chachoua A, Lafleur F, Worell CV (1988) ganciclovir treatment of gastrointestinal infections caused by cytomegalovirus in patients with AIDS. Rev Infect Dis [Suppl 10] 3:532–538
3. Dolan SA, Boesman-Finkelstein M, Finkelstein RA (1989) Inhibition of enteropathogenic bacteria by human milk whey in vitro. Pediatr Infect Dis J 8:430–436
4. Ebina T, Sato A, Umezu K, Ishida N, Ohyma S, Oizumi A, Aikawa K, Katagiri S, Katsushima N, Imai A, Kitaoka S, Suzuki H, Konno K (1985) Prevention of rotavirus infection by oral administration of cow colostrum containing antihuman rotavirus antibody. Med Microbiol Immunol 174:177–185
5. Gazzard BG (1990) Practical advice for the gastroenterologist dealing with symptomatic HIV disease. Gut 31:733–755
6. Heise W, Mostertz P, Arasteh K, Skörde J, L'age M (1988) Gastrointestinale Befunde bei der HIV-Infection. Dtsch Med Wochenschr 113:1588–1593
7. Hilpert H, Gerber H, Peyer ED, Nussle D (1974) Gastrointestinal passage of bovine anti-*E. coli* milk immunglobulins in infants. Nestle Res News 7:143–138
8. Kim KS, Dunn K, McGeary SA, Stiehm ER (1984) Efficacy of orally administered immune serum globulin against type III group B streptococcal colonization and systemic disease in an infant rat model. Pediatr Res 18:1329–1331
9. Lewis-Jones DI, Reynolds GJ (1983) A suggested role for precolostrum in preterm and sick newborn infants. Acta Paediatr Scand 72:13–17
10. Mach JP, Pahud JJ (1971) Secretory IgA, a major immunoglobulin in most bovine external secretions. J Immunol 106:552–563
11. McClead RE, Gregory SA (1984) Resistance of bovine colostral anti-cholera toxin antibody to in vitro and in vivo proteolysis. Infect Immun 44:474–478
12. Mietens C, Keinhorst H (1979) Treatment of infantile *E. coli* gastroenteritis with specific bovine anti-*E. coli* milk immunoglobulins. Eur J Pediatr 132:239–252
13. Nord J, Ma P, Dijohn D, Tzipori S, Tacket CO (1990) Treatment with bovine hyperimmune colostrum of cryptosporidal diarrhea in AIDS patients. AIDS 6:581–584
14. Pape JW (1988) Treatment of gastrointestinal infections. AIDS 2:161–167
15. Porter P (1972) Immunglobulins in bovine mammary secretions – quantitative changes in early lactation and absorption. Neonatal Calf Immunol 23:225–237
16. Sachs MK, Dickinson GM (1989) Intestinal infections in patients with AIDS. Postgrad Med 85:309–314
17. Schrager LK (1988) Bacterial infections in AIDS patients. AIDS 2:183–189
18. Seto A, Okabe T, Sosaki N, Ito Y (1976) Opsonic activity and O-agglutinins against *Escherichia coli* in bovine colostrum. Am J Res 37:635–638
19. Stephan WQ, Dichtelmüller H, Lissner R (1990) Antibodies from colostrum in oral immunotherapy. J Clin Chem Clin Biochem 28:19–23
20. Tzipori S, Robertson D, Chapmann C (1986) Remission of diarrhoea due to cryptosporidiosis in an immunodeficient child treated with hyperimmune bovine colostrum. BMJ 293:1276–1277
21. Ungar BLP, Ward DJ, Fayer R, Quinn CA (1990) Cessation of *Cryptosporidium*-associated diarrhoea in an acquired immunodeficiency syndrome patient after treatment with hyperimmune bovine colostrum. Gastroenterol 98:486–489
22. Zinkernagel RM, Colombini A (1975) Passive oral immunization with bovine immunoglobulins: enteroparhogenetic *Escherichia coli* from infants and bovine anti-*E. coli* lactoserum assayed in the rabbit ileal loop model. Med Microbiol Immunol 162:1–7

Received: January 20, 1992
Returned for revision: February 27, 1992
Accepted: July 7, 1992

Dr. A. Plettenberg
Allgemeines Krankenhaus St. Georg
HIV-Ambulanz
Lohmühlenstrasse 5
W-2000 Hamburg 1, FRG

Addendum

After this manuscript had been accepted for publication in the *Clinical Investigator*, an article entitled "Treatment of diarrhoea in human immunodeficiency virus-infected patients with immunoglobulins from bovine colostrum" by Rump et al. appeared in this journal [Clin Investig (1992) 70:588–594]. Although three of us are mentioned as coauthors, none of us knew that the manuscript had been submitted for publication by Rump et al. until the article had appeared. Seven of our cases were included in the publication by Rump et al. without our knowledge.

A. Plettenberg, A. Stoehr, H.J. Stellbrink, H. Albrecht, W. Meigel