GASTROENTEROLOGY 2004;127:630–634

CASE REPORT

Butyrate as an Effective Treatment of Congenital Chloride Diarrhea

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Background & Aims: **Many therapeutic attempts have demonstrated to be ineffective in reducing the severity of congenital chloride diarrhea and its long-term complications. The short-chain fatty acid butyrate stimulates intestinal water and ion absorption through a variety of mechanisms, including the activation of a parallel Cl^-/butyrate and Na^+/H^+ exchanger. In this case report, we report the therapeutic efficacy of butyrate on an 11-year-old patient affected by congenital chloride diarrhea.** ***Methods:*** **The efficacy of increasing doses of oral butyrate (from 50 to 100 mg/kg/day) was investigated through the daily evaluation of stool volume, bowel movements, fecal incontinence, serum, and stool electrolytes concentrations. The modifications in transepithelial intestinal ion transport elicited by butyrate were examined by rectal dialysis study.** ***Results:*** **A butyrate dose of 100 mg/kg/day induced a normalization of stool pattern and of serum and fecal electrolytes concentration. The rectal dialysis study demonstrated a proabsorptive effect induced by butyrate on Na^+, Cl^-, and K^+ intestinal transport. Butyrate therapy was well tolerated during the entire 12-month observation period, and the stool pattern and fecal and serum ion concentrations remained stable within the normal ranges. No clinical adverse events or episodes of dehydration requiring hospital care were observed.** ***Conclusions:*** **Butyrate could be effective in treating congenital chloride diarrhea. It is easily administered, useful in preventing severe dehydration episodes, and may be a promising therapeutic approach for a long-term treatment in this rare and severe condition.**

Congenital chloride diarrhea (CLD) (Online Mendelian Inheritance in Man no. 214700) is an inherited intestinal electrolyte transport disorder transmitted in an autosomal recessive manner because of mutations of the solute carrier family 26, member 3 (SLC26A3) gene. This gene is located near the cystic fibrosis transmembrane conductance regulator gene.[1,2] The disease is characterized by persistent, life-long intestinal Cl^- malabsorption, deriving from a reduced activity of the Cl^-/HCO_3^- exchanger.[1,2] The main clinical feature is prenatal onset of watery diarrhea, which may complicate pregnancy by inducing premature birth and polyhydramnios.[3,4] However, the overall clinical picture and outcome could range from severe neonatal disease, with fatal hypoelectrolytemia and dehydration, to a relatively mild chronic form, which may remain undiagnosed for a long time.[2,5] Early diagnosis of this condition is necessary in infancy because hyponatremic episodes may result in mental and psychomotor impairment and the chronic contraction of the intravascular space leading to renal dysfunction and gout.[5–7] In patients with CLD, supplementation therapy with NaCl/KCl is essential in reducing the risk of severe dehydration. Immediately after diagnosis, this therapy should be started and accurately monitored to ensure that the increasing body weight requirements are being met.[8] Unfortunately, supplementation therapy cannot limit the severity of diarrhea as in other therapeutic approaches, including acetazolamide and cholestyramine.[9,10] A proton pump inhibitor, i.e., omeprazole, was proposed for CLD treatment for its ability to reduce Cl^- gastric secretion,[11] but it was unsuccessful in other reported cases of CLD-affected children.[8] Additionally, this therapy is costly and unsuitable for life-long treatment.[12]

Recently, the role of amylase-resistant starch has been increasingly recognized for the management of diarrheal diseases.[13–15] On reaching the colon, amylase-resistant starch are fermented by resident bacteria into the short-chain fatty acids (SCFAs), including acetate, propionate, and butyrate.[16] As already shown, SCFAs have a great capacity for stimulating ion and

Abbreviations used in this paper: CLD, congenital chloride diarrhea; SLC26A3, solute carrier family 26, member 3 gene; SCFAs, short-chain fatty acids.

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0016-5085/04/$30.00
doi:10.1053/j.gastro.2004.03.071

water absorption; they provide energy and induce a trophic effect on both colonic and small bowel mucosa.[16–19] Moreover, it has been shown that SCFAs, particularly butyrate, are avidly absorbed by the intestinal mucosa and that this process is responsible for the transport of Na^+ and Cl^- through different mechanisms, primarily by the stimulation of an electroneutral NaCl absorptive mechanism activated by parallel Cl^-/butyrate and Na^+/H^+ exchanger and secondarily by up-regulation of the Na^+/H^+ and Cl^-/HCO_3^- exchangers.[20] Finally, butyrate is able to limit Cl^- secretion, inhibiting the Na^+-K^+-$2Cl^-$ cotransporter activity.[21,22] The important regulatory role of SCFAs on fluid and electrolyte absorption has led to the hypothesis that butyrate treatment could reduce diarrhea in CLD patients. In the following case study, a CLD-affected child is treated with an oral formulation of butyrate (1) to limit water and ion fecal losses, (2) to determine a positive electrolyte balance, and (3) to reduce diarrhea severity.

Case Report

An 11-year-old boy was admitted to our department because of a history of recurrent abdominal subocclusions and chronic watery diarrhea. He was the third child of unrelated parents from southern Italy. His 2 siblings were both healthy. Dilated bowel loops and polyhydramnios were detected by ultrasound during pregnancy. After 36 weeks of gestation, the baby was delivered by caesarean section. Two days after birth, the infant underwent surgery for a suspected intestinal obstruction. Subsequently, he developed watery diarrhea with recurrent metabolic alkalosis episodes, requiring hospitalization for intravenous liquid and electrolytes replacement. The median hospital stay rate since his first hospitalization was 25 days per year. Most bowel movements were associated with urgency and incontinence. The child could not regularly attend school as a result of recurrent episodes of diarrhea causing a variety of disruptions of his class. He was presented for admission to our hospital with watery diarrhea associated with a daily fecal incontinence, abdominal distension, mild dehydration, and metabolic alkalosis. His serum electrolytes were K^+ 4.3 mEq/L, Na^+ 128 mEq/L, and Cl^- 93 mEq/L. His weight (38.600 kg) and height (157.2 cm) were within the normal range for his age. An upper gastrointestinal endoscopy, small bowel barium follow-through study, anorectal and antroduodenal manometry, lactose breath hydrogen test, aldosterone, and renin and vasoactive intestinal polypeptide serum concentration all proved to be normal. His fecal Cl^-, Na^+, and K^+ concentrations were 164, 68, and 56 mEq/L, respectively, resulting in fecal cationic gap and Cl^- concentration diagnostic for CLD (fecal Cl^- content >90 mmol/L and cationic gap F - $Na^+ + K^+ < Cl^-$).[8] The diagnosis was further confirmed by molecular analysis showing 2 mutations in the SLC26A3 gene.

Materials and Methods

The Ethics Committee of our University approved the study protocol. Informed consent was obtained from the patient's parents. During the entire length of the study, the child was examined as an outpatient and received oral NaCl/KCl supplementation and continued with a normal diet. The butyrate treatment was started at 50 mg/kg/day, administered in 2 doses for 1 week. An increased dosage of 25 mg/kg/day each consecutive week was planned. A commercially available oral formulation of gastric-resistant capsules was used. The oral formulation contained calcium and magnesium butyrate salts (Butyric acid complex; Natur Bio Care, Legnago, Italy). Baseline patient data were recorded daily throughout the week before the butyrate trial and were taken as representative of the usual clinical and laboratoristic pattern of the child. Each measurement of stool volume, number of bowel movements, stool consistency (determined by an apposite score: 1 = formed, 2 = loose, 3 = semiliquid, 4 = liquid), episodes of fecal incontinence, and serum and stool electrolyte concentrations, as in the week before butyrate trial, were made daily during the weeks in which the butyrate therapy had taken place. Moreover, the hypothesized effects of butyrate on ion intestinal transport were verified in vivo by using the rectal dialysis technique, which was performed both at baseline and as soon as clinical and/or laboratory improvements were observed. In brief, a dialysis bag (1.5 inches of length) containing the isotonic electrolyte solution 20 mmol/L KCl, 30 mmol/L $NaHCO_3$, and 110 mmol/L NaCl was inserted into the rectum and left for 30 minutes. Afterward, the bag was removed and reweighed. The Na^+ and K^+ concentrations were measured by flame photometry and Cl^- using a chloridometer. The changes observed in electrolyte content were calculated and assumed to represent net fluxes (either absorptive or secretive) as reported previously.[23]

Results

During butyrate therapy, a progressive reduction was observed to normal values in the number of bowel movements and stool volume (Figure 1). Simultaneously, butyrate improved stool consistency (median daily score fell from 3.8 at baseline to 1.8 at the dose of 100 mg/kg/day) as well as reduced fecal incontinence (incidences numbering 3 days a week, in contrast to daily episodes prior to butyrate treatment, as described previously). At a dose of 100 mg/kg/day, a virtual normalization of stool pattern was obtained,

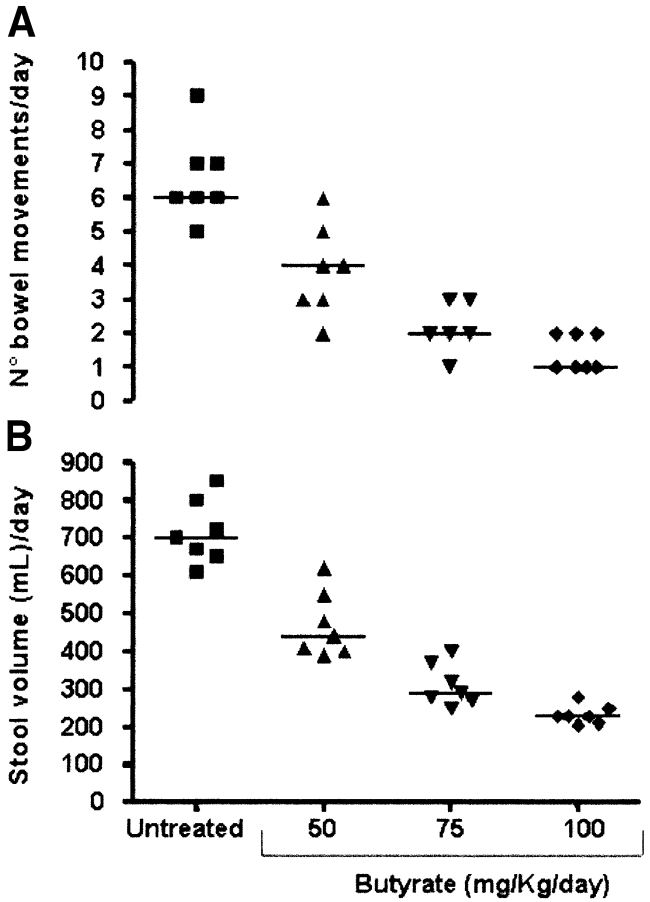

Figure 1. Clinical efficacy of butyrate therapy in the child with congenital chloride diarrhea. Daily number of bowel movements (*A*) and stool volume (*B*) during the first week of baseline data collection and the subsequent 3 weeks of treatment with increasing doses of butyrate. *Horizontal lines* represent median values of each study week. ■, untreated; ▲, 50; ▼, 75; ◆, 100 butyrate (mg/kg per day).

making higher butyrate doses unnecessary. As shown in Figure 2, a reduction of fecal electrolyte losses (determined on 1 stool sample per day) was also obtained. Serum electrolytes had already normalized in the first week of butyrate therapy (at the dose of 50 mg/kg/day). In accordance with the clinical and laboratory results, the rectal dialysis study showed a proabsorptive effect elicited by butyrate on Na^+, Cl^-, and K^+ intestinal transport (Figure 3). Following the initial trial of 3 weeks, the patient continued butyrate therapy with a dosage of 100 mg/kg/day for the next 12 months, absent of any reported adverse events. Serum electrolyte concentrations remained stable within the normal range, and no episodes of dehydration (requiring hospital care for parenteral fluid infusion) were observed during this follow-up period. Finally, for the first time, the child could attend school regularly for the entire scholastic year, with his social activities becoming normalized.

Discussion

To our knowledge, this is the first therapeutic trial using butyrate on a patient affected by CLD. The results of this investigation show dose-dependent effects of butyrate in reducing stool volume, bowel movements, and fecal incontinence. Furthermore, rectal dialysis study

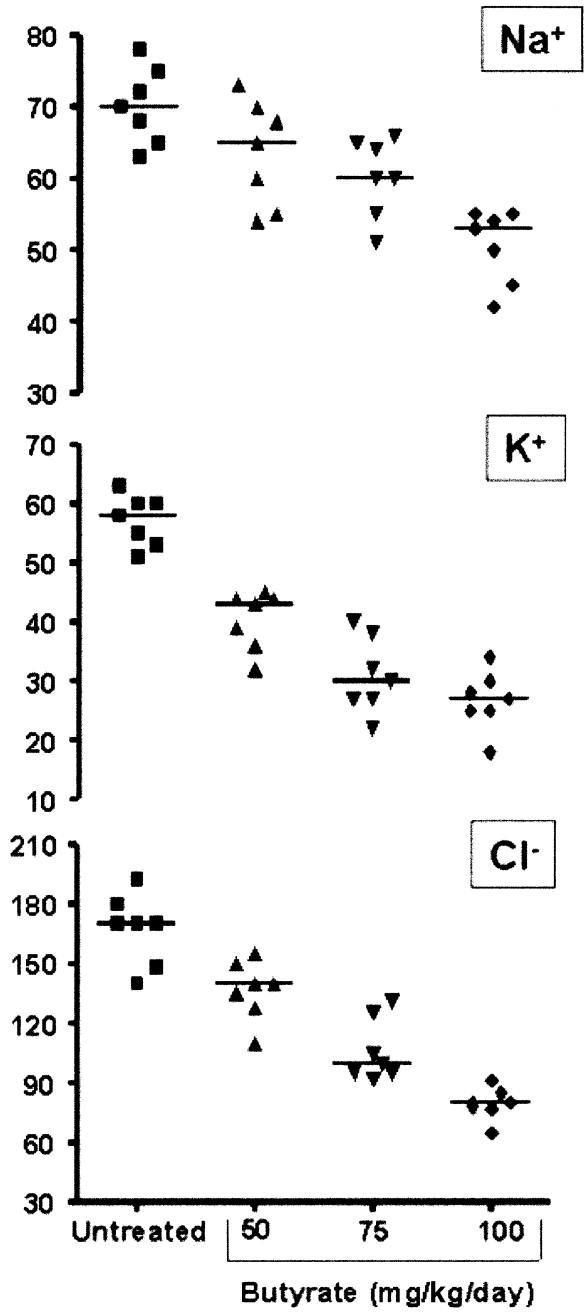

Figure 2. Effect of butyrate therapy on fecal electrolyte content. Fecal electrolyte concentrations (mEq/L) during the first week of baseline data collection and the subsequent 3 weeks of treatment with increasing doses of butyrate. Ion concentrations of 1 stool sample per day are reported. *Horizontal lines* indicate median values of each study week. ■, untreated; ▲, 50; ▼, 75; ◆, 100 butyrate (mg/kg per day).

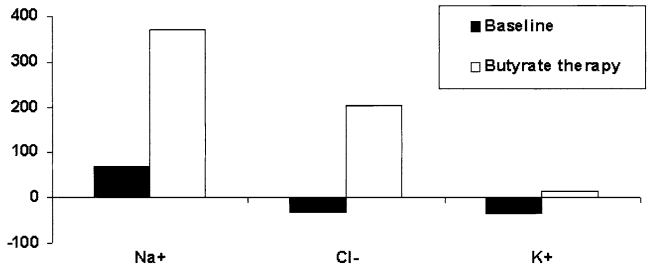

Figure 3. Rectal dialysis study. Intestinal electrolyte transport before (baseline) and during butyrate therapy (at 100 mg/kg/day) in the child affected by congenital chloride diarrhea.

demonstrated in vivo an effective up-regulation of ion absorption, which in turn could be responsible for the observed butyrate clinical effects. These results paralleled the data from the measurement of serum and fecal electrolyte contents, indicating a positive electrolytes and water balance. Through the reduction of the chronic Cl^- deficiency and intravascular volume contraction, butyrate therapy could limit the risk of severe dehydration episodes and avoid end-stage renal disease.

It has been already shown that SCFAs are absorbed along the entire length of human intestine, inducing water and ion absorption.[20,24,25] The therapeutic effects observed in our patient could be related, at least in part, to the stimulation of the Cl^-/butyrate exchanger activity.[18–20] In addition, it has been shown that butyrate is also able to inhibit both basal and adenosine 3′,5′-cyclic monophosphate–stimulated Cl^- secretion in a dose-dependent manner.[21,22] Finally, the trophic effects elicited by SCFAs on intestinal mucosa (mediated through circulatory, hormonal, and neural mechanisms) could contribute to improvement in diminishing severity of diarrhea in the CLD patient.[16]

During the entire length of the study on oral butyrate (up to the dose of 100 mg/kg/day), compliance was excellent, the oral formulation was safe, and no side effects or any untoward modifications in laboratory data were reported. The long-term beneficial effects of SCFAs were confirmed by both well-known therapeutic use of many gastrointestinal disorders and by their physiologic roles in providing energy-yielding substrates and regulating intestinal mucosa growth.[16,26] In fact, butyrate leads to growth arrest and differentiation and induces apoptosis in tumor cell lines, lowering the risk of colorectal cancer.[16,26] SCFA therapy has also shown benefits in reducing colonic inflammation and alleviates diarrhea associated with inflammatory bowel diseases, diversion colitis, and other intestinal inflammatory conditions.[27,28] Furthermore, SCFAs are able to limit intestinal fluid secretion because of severe gut infections[17] or during enteral feeding.[29] Our results in the CLD child are encouraging and could provide the basis for further studies to determine whether this treatment could be as effective in infants or in children with more severe forms of the disease. Butyrate therapy also results in cost saving through the reduction in necessity of hospital care.

References

1. Kere J, Lohi H, Hoglund P. Genetic disorders of membrane transport. Congenital chloride diarrhea. Am J Physiol 1999;276:G7–G13.
2. Makela S, Kere J, Holmberg C, Hoglund P. SLC26A3 Mutations in congenital chloride diarrhea. Hum Mutat 2002;20:425–438.
3. Gamble JL, Fahey KR, Appleton J, MacLachalan E. Congenital alkalosis with diarrhea. J Pediatr 1945;26:509–518.
4. Darrow DC. Congenital alkalosis with diarrhea. J Pediatr 1945; 26:519–532.
5. Holmberg C. Congenital chloride diarrhea. Clin Gastroenterol 1986;3:583–602.
6. Holmberg C, Perheentupa J, Pasternack A. The renal lesion in congenital chloride diarrhea. J Pediatr 1977;91:738–743.
7. Nuki G, Watson ML, Williams BC, Simmonds HA, Wallace RC. Congenital chloride losing enteropathy associated with tophaceous gouty arthritis. Adv Exp Med Biol 1991;309A:203–208.
8. Hoglund P, Holmberg C, Sherman P, Kere J. Distinct outcomes of chloride diarrhoea in two siblings with identical genetic background of the disease: implications for early diagnosis and treatment. Gut 2001;48:724–727.
9. Clark EB, Vanderhoof JA. Effect of acetazolamide on electrolyte balance in congenital chloridorrhea. J Pediatr 1997;91:148–149.
10. Brocklehurst JR, Walker AC. Cholestyramine in treatment of congenital chloride diarrhoea. Med J Aust 1978;1:504–505.
11. Aichbichler BW, Zerr CH, Santa Ana CA, Porter JL, Fordtran JS. Proton-pump inhibition of gastric chloride secretion in congenital chloridorrhea. N Engl J Med 1997;336:106–109.
12. Laine L, Ahnen D, McClain C, Solcia E, Walsh JH. Potential gastrointestinal effects of long-term acid suppression with proton pump inhibitors. Aliment Pharmacol Ther 2000;14:651–668.
13. Ramakrishna BS, Venkataraman S, Srinivasan P, Das P, Young GP, Binder HJ. Amylase-resistant starci plus oral rehydration solution for colera. N Engl J Med 2000;342:308–313.
14. Alam NH, Meier R, Sarkar SA, Bardhan PK, Fuchs GJ, Schneider H, Mahalanabis D, Gyr K. International Center for Diarrhoeal Disease Research. Efficacy of a solubile fiber supplemented oral rehydration solution in the treatment of acute non-cholera diarrhea in children. Gastroenterology 1997;112:A2.
15. Alam NH, Ashraf H. Treatment of infectious diarrhea in children. Pediatr Drugs 2003;5:151–165.
16. Topping DL, Clifton PM. Short-chain fatty acids and human colonic function roles of resistant starch and nonstarch polysaccharides. Physiol Rev 2001;81:1031–1064.
17. Rabbani GH, Albert MJ, Rahman H, Chowdhuri AK. Short-chain fatty acids inhibit fluid and electrolyte loss induced by cholera toxin in proximal colon of rabbit in vivo. Dig Dis Sci 1999;44: 1547–1553.
18. Binder HJ, Mehta P. Short-chain fatty acids stimulate active sodium and chloride absorption in vitro in the rat distal colon. Gastroenterology 1989;96:989–996.
19. Sellin JH, De Soigne R. Short chain fatty acids have polarized effects on sodium transport and intracellular pH in rabbit proximal colon. Gastroenterology 1998;114:737–747.
20. Kunzelmann K, Mall M. Electrolyte transport in the mammalian colon. Mechanism and implications for disease. Physiol Rev 2002;82:245–289.
21. Mathews JB, Hassan I, Meng S, Archer SY, Hernjez BJ, Hodin RA.

Na-K-Cl cotransporter gene expression and function during enterocyte differentiation. Modulation of Cl^- secretory capacity by butyrate. J Clin Invest 1998;101:2072–2079.
22. Resta-Lenert S, Truong F, Barrett KE, Eckmann L. Inhibition of epithelial chloride secretion by butyrate: role of reduced adenylyl cyclase expression and activity. Am J Physiol 2001;281:C1837–C1849.
23. De Curtis M, Guandalini S, Fasano A, Saitta F, Ciccimarra F. Diarrhoea in jaundiced neonates treated with phototherapy: role of intestinal secretion. Arch Dis Child 1989;64:1161–1164.
24. Schmitt MG, Soergel KH, Wood CM. Absorption of short fatty acids from the human jejunum. Gastroenterology 1976;70:211–215.
25. Mush MW, Bookstein C, Xie Y, Sellin JH, Chang EB. SCFA increase intestinal Na absorption by induction of NHE3 in rat colon and human intestinal C2/bbe cells. Am J Physiol Gastrointest Liver Physiol 2001;280:G687–G693.
26. Mortensen PB, Clausen MR. Short-chain fatty acids in the human colon: relation to gastrointestinal health and disease. Scand J Gastroenterol 1996;31:132–148.
27. Haring JM, Soergel KH, Komorowski RA, Wood CM. Treatment of diversion colitis with short-chain fatty acids irrigation. N Engl J Med 1989;320:23–28.
28. Cheppach W, Sommer H, Kirchner T, Pagenelli GM, Batram P, Christl S, Richter F, Dusel G, Kasper H. Effect of butyrate enemas on the colonic mucosa in distal ulcerative colitis. Gastroenterology 1992;103:51–56.
29. Bowling TE, Raimundo AH, Grimble GK, Silk DBA. Reversal by short-chain fatty acids of colonic fluid secretion induced by enteral feeding. Lancet 1993;342:1266–1268.

Received December 30, 2003. Accepted March 25, 2004.

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