Short-Chain Fatty Acids Improve Clinical, Pathologic, and Microbiologic Features of Experimental Shigellosis

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Because of the metabolic and antibacterial actions of short-chain fatty acids (SCFA), their roles in modifying the clinicopathologic features of shigellosis were evaluated in a rabbit model of shigellosis. Acute colitis was induced in adult rabbits by intracolonic administration of *Shigella flexneri* 2a. After 24 h, rabbits were given 6-h colonic infusions of SCFA (acetate, propionate, n-butyrate; 60:30:40 m*M*) or SCFA-free solution (control); groups of rabbits were killed in batches of 2 or 3 animals at 24, 48, 72, and 96 h after treatment, for histologic and bacteriologic assessment. SCFA significantly reduced fecal blood and mucus and improved clinical symptoms. Histologically, SCFA significantly ($P < .01$) reduced mucosal congestion, cellular infiltration, and necrotic changes. SCFA also significantly ($P < .05$) reduced the number of shigellae in the colon. No such improvements occurred in the control group. SCFA may be useful agents in improving clinicopathologic features of shigellosis and should be clinically evaluated.

Short-chain fatty acids (SCFA; acetate, propionate, and butyrate), the major anions of stool water, are produced by the anaerobic fermentation of unabsorbed carbohydrates in the colon [1–3]. Mammalian colon efficiently absorbs SCFA, which favorably affect colonocyte functions by various mechanisms including energy supply [4–6], salt and water absorption [7–9], epithelial growth [10], protein turnover [11], and intestinal blood flow and oxygen consumption [12, 13]. Low concentrations of colonic SCFA have been demonstrated in patients with inflammatory bowel diseases including ulcerative colitis, diversion colitis, and sigmoiditis [14, 15], and these disorders were successfully treated with SCFA [16–20]. SCFA, particularly acetate and formate, have been shown to possess antimicrobial properties against enteric pathogens including *Shigella* [21]. All these observations suggest that SCFA might be useful in modifying the clinical and the pathologic characteristics of shigellosis. Although treatment with an effective antibacterial agent inhibits fecal excretion of *Shigella* in humans, these drugs do not stimulate mucosal healing; the dysenteric symptoms often persist and may lead to residual complications [22]. In contrast, SCFA have the potential to provide both antiinflammatory and antibacterial effects which might act alone or as an adjunct to an antimicrobial agent, producing a better clinical response. Moreover, SCFA are dietary derivatives—safe, inexpensive, and devoid of the undesirable effect of drug-resistance. Nevertheless, very little is known about the effects of SCFA on bacterial diarrheas including shigellosis. In this study, we have therefore evaluated the effects of SCFA in a rabbit model of shigellosis by specifically examining the clinical, pathologic, and bacteriologic characteristics.

Materials and Methods

Adult rabbit model of shigellosis. In this study, the effects of SCFA were evaluated in an adult rabbit model of shigellosis recently described by Rabbani et al. [23]. In brief, acute colitis was induced in adult, nonstarved rabbits by intracolonic inoculation with *Shigella flexneri* 2a (10^7/mL) into the proximal colon after partially ligating the cecum (cecal bypass). A plastic cannula was placed in situ into the colonic segment for the administration of test solutions and for sampling colonic contents.

After bacterial inoculation, animals were returned to their cages and observed for the development of diarrhea during the next 24 h. Clinical and bacteriologic parameters were observed as described earlier [23], including body weight, rectal temperature, daily stool culture for shigellae, stool microscopy, urine analysis, and total white blood cell count with differential counts.

Treatment with SCFA. The *Shigella*-inoculated rabbits that developed symptoms of acute colitis (loose stool with mucus and blood) during a 24-h postinoculation period (pretreatment) were randomly assigned to an SCFA-treatment group or to a control group by using a table of random numbers. A total of 120 rabbits

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The protocol for this study has been approved by the Animal Ethics Experimentation Committee of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B).

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were inoculated, of which 104 (87%) developed clinical dysentery; 54 were treated with SCFA, and 50 served as controls. The treatment began 24 h after the bacterial challenge and continued through 120 h. The choice of a 24-h pretreatment period was based on our previous observations indicating development of acute colitis within that period [23]. SCFA treatment solution A contained a mixture of three SCFA: 60 m*M* sodium acetate, 30 m*M* sodium propionate, and 40 m*M* sodium n-butyrate, plus 20 m*M* sodium chloride to produce an osmolality of 300 mOsm/L. The pH of the SCFA solutions was adjusted to 7.2 by titrating with 0.1 *N* NaOH. The control solution contained 150 m*M* sodium chloride, and its pH was adjusted to 7.2 by adding Tris buffer. Both solutions had identical physical appearance and were blindly coded as treatment A and treatment B to conceal the content of the solutions. The SCFA were obtained as sodium salts of reagent-grade chemicals from Sigma (St. Louis). The concentrations of the SCFA used in these experiments were the same as those used for the treatment of diversion colitis in humans [16] and similar to or lower than that found in normal stool water, except for n-butyrate, which represented almost twice the normal concentration [17]. Treatment solutions (A or B) were given by bolus infusion into the colon at a dose of 10 mL every 6 h, beginning 24 h after infection and continuing through 120 h. Each infusion was given over a 2-min period. SCFA solution was prepared each morning and used only for that day.

Autopsy. After starting SCFA treatment, each group of animals was killed in batches of 2 or 3 animals, at each of the 4 different time points (24 h, 48 h, 72 h, and 96 h) after starting SCFA treatment. The animals were killed by an overdose of intravenous sodium Pentothal, and the visceral tissues were taken for histologic examination.

At necropsy, the serosal surfaces of the ileum, colon, and rectum were inspected for signs of inflammation and hemorrhage. If there was any fluid accumulation in the colon, the volume was measured and samples were taken for bacteriologic and biochemical examination. The segment of intestine from ileum to rectum was resected, opened, and inspected for signs of mucosal edema, exudation, hemorrhage, ulceration, necrosis, and perforation. Segments of colon were carefully excised, and 5 specimens per rabbit from the entire inflamed area at equidistant sites were taken. Samples of the tissues were fixed in 10% neutral buffered formalin for histologic examination. The tissue sections were labeled and read by a pathologist without prior knowledge of the experimental conditions.

Bacteriology. Unfixed colonic tissue was used for quantitative bacteriology. Serial 10-fold dilutions of colonic content (0.5 g) and mucosal homogenate (0.5 g) were plated on MacConkey's and *Salmonella-Shigella* agar for quantification of *Shigella.* Colony-forming units (cfu) were expressed as number per gram of tissue or colonic contents. Colonies were identified biochemically and confirmed serologically by slide agglutination with *S. flexneri* 2a antiserum (Difco, Detroit).

In vitro antibacterial effects of different SCFA on *S. flexneri* 2a were assessed by the tube dilution method. Individual SCFA were serially diluted 2-fold, starting with a concentration of 60 m*M* in Mueller-Hinton broth (Gibco Laboratories, Grand Island, NY), and then inoculated with 20 μL of a suspension containing 10^6/mL of *S. flexneri* 2a grown in Mueller-Hinton broth for 6 h and incubated overnight at 37°C. Bacterial growth in each tube was quantified by plating serial dilutions on MacConkey's agar, counting the colonies after overnight incubation at 37°C, and expressing them as cfu per milliliter (cfu/mL).

Macroscopic grading of colonic inflammation. At autopsy, the severity of colonic inflammation was assessed according to a modified method of sigmoidoscopic assessment of colitis described by Harig et al. [16]. Immediately after sacrifice (within 3-5 min), the colonic mucosa was examined by a high-power lens (Medical Nikor, 22×; Nikon Corporation, Tokyo) fitted with a 35-mm single-lens reflex camera, and several macrophotographs were taken on a high-resolution transparency film. Enlarged views of the macrophotographs were reviewed by another observer, and a macroscopic score of inflammation was established [16]. Each of the five abnormalities, erythema, edema, friability, granularity, and erosion, was scored as absent (grade 0), mild (grade 1), moderate (grade 2), or severe (grade 3). The sum of the scores for an individual rabbit was considered to be a macroscopic index, with a range of 0–10. The score (mean ± SD) for each treatment group at each time point was based on a weighted mean of the individual scores.

Histopathologic grading of the severity of colonic inflammation. Coded autopsy specimens were examined by a light microscope by an experienced pathologist (M.M.I.). During the histologic evaluation, the severity of inflammation was graded according to a semiquantitative scoring system described by Karnell et al. [24] in a monkey model of shigellosis. According to this system, autopsy specimens are classified into normal, mild, moderate, and severe colitis, depending on the severity of inflammatory changes.

Mild colitis was defined as (1) an increased number of polymorphonuclear leukocytes in the lamina propria, (2) with or without slight edema in the lamina propria, and (3) slight goblet cell depletion.

Moderate colitis was present when (1) the surface epithelium was eroded, (2) an increased number of inflammatory cells was seen in the lamina propria, (3) a moderate to severe depletion of goblet cell population was seen, and (4) an occasional hemorrhage with or without vasculitis in the lamina propria and submucosal layers was noted.

Severe colitis was characterized by broad surface erosions and superficial ulcerations accompanied by substantially increased numbers of neutrophils in the lamina propria, crypt abscess formation, more pronounced goblet cell depletion (with or without deep ulceration), and involvement of the underlying muscle coat.

Colitis index. For assessing the severity of acute colitis, a colitis index was established by assigning numeric scores according to the severity of inflammation: Thus, mild colitis was scored as 1.0, moderate colitis as 2.0, and severe colitis as 3.0 points. In individual rabbits, the colitis index was defined as the ratio between the sum of the histologic scores and the number of autopsy specimens. In each treatment group, the colitis index was defined as the ratio between the sum of the scores and the total number of autopsies in that particular group.

Coefficient of improvement. A numeric indicator of improvement of colitis (coefficient of improvement) was calculated based on the percent changes of colitis index values at each posttreatment period in relation to pretreatment value for each treatment group. Coefficient of improvement = colitis index [pretreatment] × 100/ colitis index [after treatment].

Statistics. Statistical comparisons of the severity of colitis in the two groups were made by either Student's t test or χ^2 test.

Results

Characteristics of the dysenteric illness before treatment. Prior to treatment, there were no significant differences in any of the clinical characteristics with regard to age, gender, body weight, and intensity of illness between the SCFA-treated rabbits and the control group (table 1). Within 24 h of challenge with *S. flexneri* 2a, 104 (87%) animals developed clinical signs and symptoms of acute rabbit shigellosis characterized by frequent passage of a thick liquid stool mixed with large amounts of mucus and occasional blood. Signs of bacterial infections were observed, including a rise in rectal temperature and leukocytosis. The rabbits appeared ill, were anorectic, and had marginal loss of body weight during the 24 h postchallenge period.

At 24 h, autopsy examinations revealed that inoculation of the colon with virulent *S. flexneri* 2a consistently resulted in an accumulation of exudate in the lumen and development of acute colitis. Abnormalities were confined to the colon; the colonic wall was thickened, and the mucosa appeared swollen, injected, and granular, with localized or patchy areas of hemorrhage.

Histologically, the mucosa demonstrated an acute colitis that was markedly different from an uninfected, normal colon. The lesions were characterized by severe, acute, hemorrhagic inflammation in the mucosa and submucosal layers; there was pronounced edema, occasional thrombosis in blood vessels, and active vasculitis in the submucosa. The surface epithelium was eroded in many places, leading to formation of superficial ulcers. The lamina propria was congested, edematous, and infiltrated with numerous polymorphonuclear leukocytes. In some areas, there were aggregates of fibrinopurulent exudate, tissue debris, and macrophages, which often appeared as a pseudomembrane adherent to the damaged mucosa. In some instances, there were focal hemorrhages and inflammation in the muscle layer, and the underlying serosa appeared to be inflamed (serositis).

Clinical evaluation of treatment effects. Changes in the clinical characteristics induced by treatment with SCFA in the *Shigella*-infected rabbits are given in table 2. Before SCFA treatment, *Shigella*-inoculated animals in both groups had developed acute dysenteric illness characterized by loss of body weight, passage of loose stools with blood and mucus, and presence of fecal leukocytes and erythrocytes in the stool. However, within the first 48 h of SCFA treatment, most animals in the treatment group showed significant clinical improvement versus animals in the control group. These improvements were reflected by a significant reduction in the number of fecal inflammatory cells, a change in the physical appearance of the stool, and an absence or reduction of fecal blood and mucus. SCFA treatment also significantly ($P < .05$) prevented loss of body weight, rise of rectal temperatures, and development of leukocytosis in the infected rabbits. During the second 48 h of observation (i.e, 72–120 h after inoculation), the SCFA-treated rabbits continued to improve clinically, as indicated by a further improvement of the clinical characteristics listed in table 2. By the end of 120 h, most SCFA-treated animals had recovered from the dysenteric illness, with only 8% and 17%, respectively, having visible blood and mucus in the stool.

Table 1. Clinical characteristics of *Shigella flexneri* 2a–infected rabbits before starting SCFA treatment (24 h after inoculation).

Characteristics	Control	SCFA-treated
No. of rabbits	54	50
Age (weeks)	22.1 ± 3.1	22.8 ± 4.1
Body weight (kg)	2.6 ± 0.5	2.8 ± 0.4
Rectal temperature (°C)	40.2 ± 1.9	39.8 ± 1.6
Body weight loss (%)	3.2 ± 0.2	2.5 ± 0.3
Blood in stool (%)	66	61
Mucus in stool (%)	93	96
No. of fecal leukocytes/hpf	30 ± 11	36 ± 13
No. of fecal erythrocytes/hpf	16 ± 5	13 ± 3
Isolation of *S. flexneri* 2a from stool (%)	100	100
Total leukocyte count ($\times 1000/mm^3$)	15.8 ± 3.6	13.8 ± 2.9
Serum specific gravity	1.0242 ± 0.002	1.0231 ± 0.001

NOTE. SCFA, short-chain fatty acids. Data are mean ± SD or percentages. All rabbits had dysentery. hpf = high-power field.

In contrast, the control group of rabbits continued to manifest symptoms of acute dysentery during the corresponding posttreatment periods. During the observation, the animals in the control group generally appeared more sick, anorectic, and lethargic, with increasing loss of body weight.

There were 3 deaths: 1 in the treatment group within 24 h of SCFA treatment, and 2 in the control group during 24–48 h of saline treatment. The dead animals were not included in the study analysis.

Macroscopic evaluation of treatment effects. Table 3 describes the changes in the severity of colonic lesions as assessed by macroscopic score. The macroscopic scores in the SCFA-treated and control group were significantly different during all the observation periods except the first 24 h. This indicates that there was a significant ($P < .01$) and progressive improvement of the intensity of inflammation in the SCFA-treated rabbits during the observation period. There was no significant change in the mean values of macroscopic score at 24 h after starting SCFA treatment; thereafter, the macroscopic scores gradually declined from 7.8 to 1.1 in the SCFA treatment group, whereas the macroscopic scores in the control group did not change significantly (reduced from 9.4 to 6.1).

Histopathologic evaluation of treatment effects. Figure 1 shows the beneficial effects of SCFA treatments, as indicated by a favorable change in the mean values of the colitis index. During the 24-h pretreatment period, the mean ± SD colitis index was 2.75 ± 0.23, indicating a severe grade of inflammation. At 24 h after starting SCFA treatment (i.e., 48 h after inoculation), there was a marginal reduction in the colitis index in the treatment group compared with the control group

Table 2. Effects of SCFA treatment on clinical characteristics of shigellosis in adult rabbits.

Characteristics	Time (h) after starting SCFA treatment			
	24	48	72	96
No. of rabbits				
Treatment	13	13	14	12
Control	16	11	13	12
Body weight loss (%)				
Treatment	4 ± 2[a]	4 ± 1.6	5 ± 1[a]	6 ± 1.5
Control	9 ± 3	10.3 ± 3	12 ± 3	12.3
Blood in stool (%)				
Treatment	46[a]	20	8.0[a]	6.0
Control	85	81	76.0	80.0
Mucus in stool (%)				
Treatment	43[a]	26[a]	17[a]	8.0[a]
Control	86	82	78	83
Soft stool consistency (%)				
Treatment	58[a]	66[a]	75[a]	85.0[a]
Control	10	16	22	25.0
Rectal temperature (°C), mean ± SD				
Treatment	40.3 ± 0.38[a]	39.8 ± 0.1	39.6 ± 42.43[a]	38.2 ± 0.3
Control	42.8 ± 0.43	42.6 ± 0.8	41.9 ± 0.41	42.7 ± 0.4
Fecal leukocytes (no. of cells/hpf), mean ± SD				
Treatment	13 ± 6[a]	8 ± 3	5 ± 4[a]	5 ± 2
Control	40 ± 15	35 ± 10	31 ± 10	35 ± 12
Fecal erythrocytes (no. of cells/hpf), mean ± SD				
Treatment	4 ± 2[a]	3 ± 1	3 ± 2[a]	3 ± 1[a]
Control	20 ± 8	11 ± 3	8 ± 4	12 ± 3
Total leukocyte count (10^3/mm^3), mean ± SD				
Treatment	10.8 ± 3.6[a]	11.2 ± 2	8.1 ± 2.6	9.3 ± 2.1[a]
Control	12.2 ± 4.8	13.2 ± 3	13.7 ± 3.5	14.3 ± 3.1

NOTE. SCFA, short-chain fatty acids; hpf, high-power field.
[a] Statistically significant ($P < .001$ or $P < .05$) by χ^2 or Student's t test (treatment vs. control).

(2.31 ± 0.35 vs. 2.62 ± 0.32, respectively), but this difference was not statistically significant ($P < .50$). At 48 h after starting treatment, SCFA significantly reduced the colitis index in the treatment group compared with the controls (1.71 ± 0.22 vs. 2.51 ± 0.32, $P < .05$). These values indicate a mild to moderate colitis in the SCFA treatment group and persistent severe colitis in the controls. At 72 h after treatment (i.e., 96 h after inoculation), further improvement of the colonic inflammation occurred in the SCFA treatment group, as indicated by a significantly lower colitis index in the treatment group versus the control group (1.24 ± 0.21 vs. 2.49 ± 0.24, $P < .01$). This indicates that most SCFA-treated animals had mild colitis, whereas most control animals had severe colitis. After 96 h of SCFA treatment (i.e., 120 h after inoculation), the colitis index was further reduced in the treated animals compared with the controls (0.92 ± 0.10 vs. 2.33 ± 0.27, $P < .001$). At this stage, the colonic mucosa looked almost normal, as assessed by light microscopy. In contrast, there was little or no such improvement of colitis in the control group of animals.

Table 3. Changes in macroscopic scores (MS) of colitis induced by SCFA treatment in rabbit shigellosis.

Time (h) after treatment	MS (mean ± SD)		P[a]
	Control	SCFA-treated	
24	9.4 ± 3.5	7.8 ± 2.6	NS
48	8.2 ± 2.9	5.1 ± 1.0	.05
72	7.8 ± 2.1	3.2 ± 0.61	.01
96	6.1 ± 2.5	1.1 ± 0.12	.001

NOTE. SCFA, short-chain fatty acids; NS, not significant. MS of inflammation was established as described by Harig et al. [16]. Each of 5 abnormalities (erythema, edema, friability, granularity, and erosion) as scored as absent (grade 0), mild (grade 1), or severe (grade 2). Sum of scores was considered to be macroscopic index, with range of 0–10.
[a] By Student's t test, SCFA vs. control.

Coefficient of improvement. Coefficient of improvement is derived from the colitis index values; it indirectly reflects the posttreatment changes relative to the pretreatment value in each treatment group. The coefficient of improvement at 24 h after SCFA treatment was 13%, which gradually increased to 49% at 48 h, 59% at 72 h, and 72% at 96 h. In the control group, the corresponding values were significantly lower: 8% at 24 h, 10% at 48 h, 13% at 72 h, and 20% at 96 h. Thus, there was a gradual improvement of the colonic lesion by SCFA treatment, as indicated by a gradual increase in the coefficient of improvement; in the control group, there was no improvement of colitis, as indicated by little or no change in the coefficient of improvement.

Bacteriologic evaluation of treatment effects. Figure 2 shows the bacteriologic improvements after treatment with SCFA. There was a progressive reduction in the number of viable *S. flexneri* 2a in the colonic lumen and in the colonic mucosal tissue. Within 24 h of SCFA treatment, the mean number of bacteria (cfu/mL) in the tissue and the colonic lumen was reduced, but the differences between the treatment and

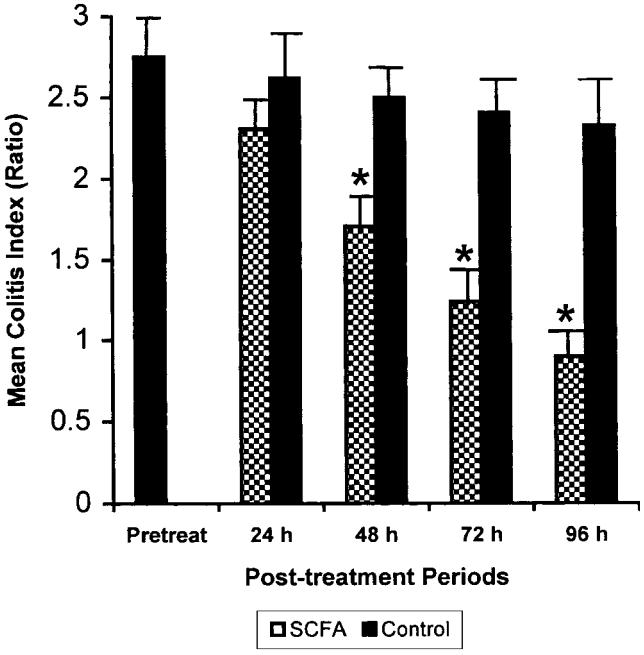

Figure 1. Effects of SCFA treatment on histologic improvement of colitis due to *S. flexneri* 2a infection in rabbits as assessed by colitis index (CI). CI is established by assigning numeric scores according to severity of inflammation: mild = 1.0, moderate = 2.0, and severe = 3.0 points. CI is defined as ratio between sum of histologic scores and total no. of autopsy specimens. Solid bars show mean CI of control rabbits; patterned bars show those of SCFA-treated rabbits. * Statistically significant *P* values (<.05 or <.01). Gradual decline of CI values in SCFA-treated rabbits indicates significant improvement of colitis; marginal decrease in CI values in control rabbits indicates no improvement.

control group were not significant. By 48 h, SCFA treatment significantly ($P < .01$) reduced the mean number of bacterial counts (cfu/mL) in both the colonic tissue and the lumen (tissue: 2.8 ± 0.32 vs. 4.1 ± 0.4; lumen: 3.1 ± 0.34 vs. 5.1 ± 0.51). At 96 h after treatment, the mean numbers of cfu/mL were significantly lower in the SCFA group than in the control group (tissue: 1.1 ± 0.03 vs. 2.8 ± 0.19; lumen: 1.3 ± 0.23 vs. 3.3 ± 0.35). Although the numbers of viable *S. flexneri* 2a in the lumen were slightly lower than those in the mucosal tissue, there was a good positive correlation ($r = 0.87$) between the log numbers of bacteria in the mucosal tissue and those in the colonic lumen.

Figure 3 illustrates the in vitro antibacterial effects (dose-response) of SCFA on *S. flexneri* 2a. There was a good dose-dependent bactericidal effect of all three individual SCFA. Acetate (60 m*M*) produced the highest inhibition, whereas n-butyrate (40 m*M*) and propionate (30 m*M*) produced lower but consistent effects.

Discussion

The rabbit model of shigellosis used in this study has been developed and reported by us recently [23]. In this model, the cecum of an adult rabbit is obstructed, and shigellae were introduced directly into the colon without any pretreatment. This procedure resulted in successful bacterial colonization of the rabbits and development of a dysenteric illness resembling human shigellosis. The characteristics of our model are different from those of the earlier models, which invariably required pretreatment with antimicrobial, antimotility, and toxic agents [24–27]. These treatments made the animals physiologically unsuitable for therapeutic studies. Unlike the previous models, the animals in our study survived longer (120 h) and showed the characteristic symptoms of shigellosis. These features allowed us to successfully use this model to evaluate the effects of SCFA by specifically examining the clinical, bacteriologic, and histopathologic characteristics.

Our study was carried out as a randomized, controlled, and observer-blinded trial, the most efficient way of evaluating the effects of clinical interventions. The clinical criteria used for assessing the effects of SCFA in rabbits were very similar to those used in humans [28]. Our results showed that before receiving treatment with SCFA, rabbits in both SCFA and control groups had comparable characteristics with regard to age, sex, body weight, intensity of infection, and severity of colitis. Within 24–48 h of starting SCFA treatment, we observed significant clinical improvement that persisted for 120 h. The response to SCFA administration in the rabbits, as indicated by

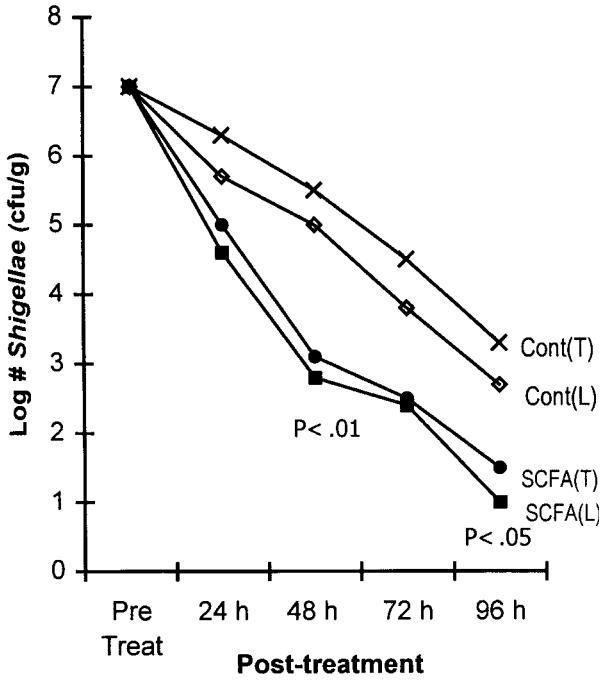

Figure 2. Antibacterial effects of SCFA treatment in experimental shigellosis in rabbits. Each point represents mean count (cfu/g) of viable *S. flexneri* 2a recovered from colonic lumen (L) or tissue (T) of SCFA-treated or control (untreated) rabbits. SCFA treatment significantly reduced bacterial counts (cfu/g) in colon compared with untreated animals at 48 h and 96 h after starting treatment.

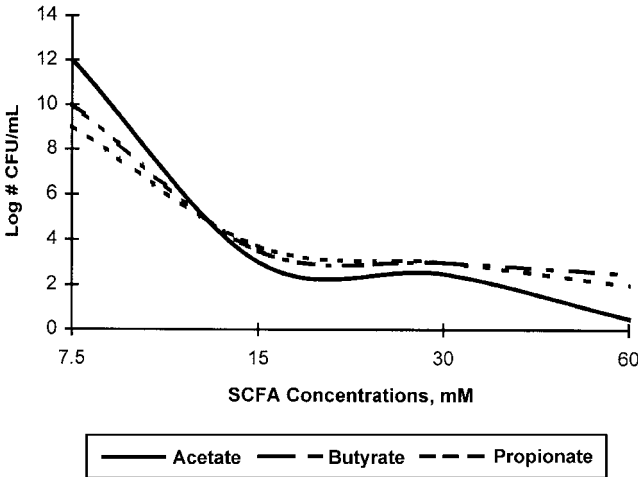

Figure 3. In vitro bactericidal effects of different SCFA on *S. flexneri* 2a. Bacteria were grown on Mueller-Hinton broth containing different SCFA at different molar concentrations and then plated on MacConkey's agar. After overnight incubation at 37°C, colonies were counted and expressed as no. of colony-forming units (cfu/mL). There is dose-dependent antibacterial effects of all 3 SCFA; 60 m*M* acetate produced highest inhibition, followed by 40 m*M* propionate and 30 m*M* n-butyrate.

the clinical and pathologic findings, was very similar to that seen in human shigellosis [29]. In our study, the most important effect of SCFA was the healing of the damaged colonic mucosa, as reflected by the values of colitis index and the macroscopic scores.

Our study indicated that SCFA may be a useful adjunct in reducing clinical severity and fecal excretion of *Shigella* organisms. In humans, the mainstay of treatment is the use of specific antimicrobial agents that can rapidly kill *Shigella* in the colon [28–30]. Nevertheless, in many instances, the clinical symptoms and the inflammatory lesions persist and often give rise to residual complications [31]. Moreover, the frequent development of drug resistance by shigellae limits the usefulness of antimicrobials in the treatment of shigellosis [30]. Unlike antibiotics, SCFA are able to repair the damaged mucosa and kill shigellae in the colon at the same time. Synergistically, these effects are likely to result in an improvement of the clinical and pathologic features of the disease.

To explain our observations, it is important to examine whether SCFA are merely correcting an artificial deficiency induced surgically in our rabbit model or affecting the pathogenic mechanisms of the disease itself. It has been shown that SCFA are not only able to improve the colonic lesions associated with surgical diversion of feces (diversion colitis, ileal pouchitis) [16, 32, 33] but can also beneficially affect natural colonic diseases, including ulcerative colitis and proctosigmoiditis [16–18]. Moreover, all conditions in which SCFA have been shown to produce an antiinflammatory and cytoprotective effect were associated with SCFA deficiency.

Reductions of fecal SCFA contents have been reported in patients with cholera [34] and *Shigella*-like invasive intestinal infections including enteroinvasive *Escherichia coli, Entamoeba histolytica,* and *Yersinia enterocolitica* [35]. SCFA deficiency has also been reported in many other intestinal disorders, including antibiotic-associated diarrhea [36] and nutritional colitis [37]. Although a similar reduction of SCFA has not yet been reported in shigellosis, several observations suggest that such a possibility cannot be excluded. In a placebo-controlled trial in children with diarrhea due to various enteropathogens including *Shigella,* we found that a 5-day course of pectin or green banana, which are rich sources of SCFA, significantly reduced volume of stool and duration of illness, and improved stool quality [38]. Similar clinical benefits were also obtained using guar gum [39] and "Isphagula" husk (personal observations), which are both fermentable fibers that act by producing SCFA in the colon. These observations support the view that SCFA may be deficient in shigellosis, which responds to exogenous supplementation of SCFA. Moreover, shigellosis has striking similarities to ulcerative colitis and, in both diseases, the pathologic lesion is most severe in the rectosigmoid area, which has the least concentrations of SCFA [40, 41]. These findings provide additional support in favor of an SCFA deficiency in the colon of patients with shigellosis. Reduction of colonic SCFA in shigellosis may also result during treatment with different antimicrobial agents, including ceftriaxone and ampicillin, which are active against the anaerobes [51, 52].

What are the mechanisms by which SCFA produce the observed healing effects in *Shigella*-induced colitis? It is likely that SCFA act not only by correcting a deficiency but also affect other possible mechanisms of the disease. It has been shown that colonic mucosal cells preferably utilize SCFA as their major source of metabolic energy; this is reduced during active colitis due to impaired oxidation of SCFA [9, 42]. The observed antiinflammatory effects of SCFA in our study, together with those reported in other studies [16–19], indicate that an increased availability of SCFA in the colonic lumen probably enhances the intracellular concentration and oxidation of SCFA, despite the metabolic defect. SCFA have been shown to stimulate proliferation and differentiation of colonocytes in experimental animals [43] and in cancer cells [44–46]. The cytoprotective effects of SCFA may be mediated by their stimulating effects on mucus release, which prevents the mucosal microenvironment from the acidic colonic contents [14, 15]. Rapid regeneration of the damaged colonic epithelium may also occur due to the stimulating effects of SCFA on mucosal transglutaminase activity, which promotes protein building through cross-linking of different proteins and inhibiting fibrinolysis [47]. Moreover, SCFA are known to dilate resistance arteries of the colon and increase blood flow and mucosal oxygen uptake [12, 13]; these mechanisms are probably involved in the rapid improvement of vascular lesions and bleeding associated with colitis due to shigellosis. Finally, it is known that SCFA, particularly butyrate, produced in the colon are well absorbed and significantly stim-

ulate colonic salt and water absorption [7–9], thus reducing the osmotic fluid load in the colon.

The colonic concentration of SCFA is considerably high, both in humans and rabbits [48, 49]. Therefore, it is important to consider why endogenous SCFA do not show the same bactericidal and colonotrophic effect obtained with the administration of relatively low concentrations of exogenous SCFA. Although a definitive answer to this question is not available now, it may be related to a complex interaction involving the colonic flora, substrate fermentation, and mucosal metabolism of SCFA under different conditions. The high concentrations of endogenous SCFA normally seen may not be present during active colitis, as observed in inflammatory bowel disease [16–19]; the oxidation of SCFA by the inflamed mucosa is impaired [9], resulting in high intraluminal concentrations of SCFA [50]; and other inflammatory mediators, including cytokines, nitric oxide, and reactive oxygen species may be involved. We have observed a good correlation between the in vivo and in vitro bactericidal effects of SCFA, suggesting that a relatively low concentration of exogenous SCFA may be considered apparently high in the face of reduced luminal concentrations and utilization of SCFA.

The observed antibacterial effects of SCFA in our study may be explained by direct action of SCFA in the lumen, while the interstitial effect may be mediated through absorption and metabolism of SCFA by the colonocytes [7, 8]. Even if the colony counts in the colonic lumen and in the mucosal tissue seem to correlate, there is still the possibility that SCFA can induce the loss of the *Shigella* invasive plasmid. This aspect needs further studies. The antibacterial actions of SCFA are consistent with the observations of Hentges [21], showing similar effects in vitro, probably mediated through high concentrations of SCFA and low pH in the media.

In conclusion, we have shown that a mixture of acetate, propionate, and butyrate given by colonic infusion in rabbits with acute shigellosis improves clinical, pathologic, and bacteriologic characteristics. These findings suggest that SCFA or their metabolic precursors (such as dietary fibers) need to be clinically evaluated for similar benefits in humans.

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