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Effect of 4^G-β-D-galactosylsucrose (lactosucrose) on fecal microflora in patients with chronic inflammatory bowel disease

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Abstract: Metabolic interaction between the intestinal microflora and the host has been suggested to play a role in the pathogenesis of chronic inflammatory bowel disease. Elemental or low-fat, low-residual diets in patients with Crohn's disease or ulcerative colitis are reported to decrease anaerobic bacteria and to change the composition of the intestinal microflora. We examined the effect of an indigestible agent, 4^G-β-D-galactosylsucrose (lactosucrose), which is selectively utilized by intestinal *Bifidobacterium*, on the composition of the intestinal microflora. After the administration of lactosucrose to two patients with Crohn's disease and five patients with ulcerative colitis for 2 weeks, significant induction of the growth of *Bifidobacterium* was observed, and significant reduction in the population level of Bacteroidaceae was seen. Bowel movements improved in four patients. The intestinal environment, estimated by measuring fecal pH, fecal levels of short-chain fatty acids and putrid products, and the urinary secretion of indican, also improved in these patients. These results suggest that lactosucrose may be useful for patients with chronic inflammatory bowel disease.

Key words: lactosucrose, Crohn's disease, ulcerative colitis, *Bifidobacterium*

Introduction

The incidence of chronic inflammatory bowel disease, i.e., ulcerative colitis and Crohn's disease, is increasing in Japan.[1] In addition to drug therapy, nutritional support is important for maintaining remission in such diseases and for improving the quality of life. The diet for patients with chronic inflammatory bowel diseases is designed to reduce fat content and fecal volume so as to prevent bowel irritation. Based on this concept, an elemental diet has been used to improve the nutritional status of patients with Crohn's disease. We have previously assessed the nutritional intake in outpatients with Crohn's disease at Kawasaki Medical School Hospital, and reported that 50%–70% of total energy was obtained from an elemental diet in these patients.[2] When the patients obtain energy mainly from the elemental diet, they are subsequently required to take a high-carbohydrate, high-protein diet with low dietary fiber. This dietary manipulation has been reported to decrease anaerobic bacteria in the intestine.[3] In patients with chronic inflammatory bowel disease, abnormal composition of the intestinal microflora has been observed,[3–7] and the potential role of the intestinal microflora in the etiopathogenesis of chronic inflammatory bowel disease has been suggested.[8–10] It has also been suggested that alterations of intestinal microflora induced by an elemental diet may be related to the relapse of chronic inflammatory bowel disease.[11,12] Therefore, interaction between the intestinal microflora and the host must be consaidered in nutritional support.

In the present study, we investigated the effect of an indigestible oligosaccharide (lactosucrose), which is selectively utilized by intestinal *Bifidobacterium*, on the intestinal microflora in Crohn's disease and ulcerative colitis patients, and found that the administration of lactosucrose significantly stimulated the growth of *Bifidobacterium* and had some effect in improving the intestinal environment in these patients.

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Materials and methods

Patients

This series comprised two patients with Crohn's disease and five patients with ulcerative colitis. All patients had the histologic changes suggestive of Crohn's disease or ulcerative colitis. The pertinent clinical data are listed in Table 1. All the patients were admitted to Kawasaki Medical School Hospital. Three patients had surgical histories. Patient 1 had had a terminal ileectomy due to stenosis of the terminal ileum 12 months before this study. Patient 2 had undergone an ileocolectomy, due to blind loop syndrome, 20 years before this study. A total colectomy was carried out in patient 5, due to acute deterioration of ulcerative colitis, 7 months before this study. Clinical and laboratory examinations showed all patients to be in the remission stage. They were treated with maintenance doses of prednisolone and/or salazosulfapyridine. These medications were not changed during the study period. Clinical and biochemical examinations revealed no recurrence of the disease in any of the patients during the period of administration of lactosucrose.

All patients took 15 g per day of high-lactosucrose syrup, containing 8.5 g lactosucrose, orally, for 14 days. The study period was 21 days; the 14 days of lactosucrose administration and observation 7 days after withdrawal of the agent. The dose and duration of administration were determined after examining the effect of lactosucrose on the intestinal microflora in healthy volunteers.[13] Lactosucrose was synthesized from lactose and sucrose by β-fructoflanocidase (from *Arthrobacter* sp. K-1), as previously described.[14] The sugar composition of the syrup is shown in Table 2.

This study was approved by the Committee of Bioethics, Kawasaki Medical School Hospital, in accordance with the World Helsinki Declaration. The subjects understood the content of the study and gave their written consent to participate in the study.

Fecal sampling and analysis

The frequency of delivery, fecal volume, properties of feces, and occult blood were monitored every day throughout the study period. Fecal sampling was done 1 day before the administration of lactosucrose, on days 7 and 14 of the study period, and again on the 7th day after lactosucrose had been withdrawn. All fecal samples were delivered into sterile anaerobic bags and were subjected to bacteriological examination, within 3 h, by the method of Mitsuoka.[15,16] The results were expressed as the log 10 of the number of bacteria per g wet weight of fecal material. Fecal moisture was calculated by subtracting fecal dry weight from fecal wet weight. The feces were mixed well, and the pH was measured by the direct insertion of a pH meter into the mixture. Total fecal nitrogen was measured by the Kjeldahl method, and fecal protein content was calculated, using a protein conversion factor from nitrogen of 6.25. Fecal putrid products were estimated by measuring the content of indoles (indole and skatole) and phenols (phenol, *p*-cresol, and 4-ethylphenol) by gas chromatography, according to the method of Yoshihara.[17]

For the measurement of the content of short-chain fatty acids (malic, succinic, lactic, formic, acetic, propionic, butyric, and valeric acids), the feces were

Table 1. Clinical findings of the seven patients in this series

Case no.	Age (years)	Sex	Diagnosis	Pathoanatomy	History of operation	Medication (per day)	Diet
1	44	Female	Crohn's disease	Ascending colon Terminal ileum	Terminal ileectomy (15 cm)	Salazosulfapyridine (1.5 g)	Elemental diet
2	57	Male	Crohn's disease Gastric ulcer	Transverse colon	Resection of blind loop of ascending colon and ileum	Salazosulfapyridine (1.5 g) Betamethasone (1 mg)	Low-residue, low-fat diet
3	17	Male	Ulcerative colitis	Sigmoid colon Rectum		Prednisolone (10 mg) Salazosulfapyridine (1.5 g) Betamethasome (1 mg)	Low-residue, low-fat diet Liquid diet
4	20	Male	Ulcerative colitis	Ascending colon Transverse colon Sigmoid colon Rectum		Prednisolone (15–30 mg) Betamethasone (1 mg)	Low-residue, low-fat diet
5	23	Male	Ulcerative colitis	Entire colon	Total colectomy	Prednisolone (5 mg) Betamethasone (1 mg)	Low-residue, low-fat diet
6	29	Male	Ulcerative colitis	Descending colon Sigmoid colon Rectum		Prednisolone (20–30 mg) Salazosulfapyridine (1.5 g) Betamethasone (1 mg)	Low-residue, low-fat diet
7	68	Female	Ulcerative colitis	Transverse colon Descending colon Rectum		Prednisolone (15 mg) Betamethasone (1 mg)	Low-residue, low-fat diet

Table 2. Sugar composition of high-lactosucrose syrup

Sugar	Percentage
Glucose	1.0
Fructose	1.5
Sucrose	5.7
Lactose	8.6
Lactosucrose	75.5
Other	7.7

diluted five times with distilled water. The pH was then adjusted to 2.5 with 0.1 N H_2SO_4. After centrifugation for 10 min at 12 000 *g*, the supernatants were filtered though Molcut II (Nihon Millipore, Tokyo, Japan) and subjected to analysis with an ion chromatoanalyzer (IC-500P; Yokogawa, Tokyo, Japan).

Urinary analysis

Indican, a breakdown product of tryptophan, reflects bacterial activity in the small bowel and colon,[18,19] Urinary indican levels of 24-h collected urine were measured by the method of Tohyama et al.,[18] with minor modifications. Five ml of diluted urine was mixed with 0.5 ml of 1% $K_2S_2O_8$, 0.5 ml of 1% thymol-95% ethanol, and 5.0 ml of 25% trichloroacetic acid in 35% HCl solution. The mixture was allowed to stand for 15 min at room temperature. The mixture was then heated at 98°C for 20 min, the lower colored layer was collected in test tubes, and glacial acetic acid was added to a volume of 4.0 ml. The optical density was determined at 540 nm against the reagent blank. For the blank, ethyl alcohol (95%) instead of thymol/alcohol was added to the diluted urine and the mixture was treated in the same way as the smaple.

Statistical analysis

All values are given as means ± SD. Bacterial counts are shown as logarithmic values per g wet feces. Each bacterial count is shown as mean ± SD obtained from the respective bacteria-positive feces. Paired-Student's *t*-test was used to determine differences in total bacterial counts before and after the administration of lactosucrose. The significance level was $P < 0.05$. The effect of lactosucrose on the growth of *Bifidobacterium* was evaluated by comparing the frequency of detection by Fisher's exact probability test. The significance level was $P < 0.05$. The Wilcoxon signed-rank test was used to determine differences in other values before and after administration of lactosucrose. *P* values of less than 0.05 were considered to be statistically significant.

Results

Effect of lactosucrose on bowel movements and laboratory findings

After the administration of lactosucrose, bowel movements, estimated by the frequency and regularity of defecation and the properties of feces, were improved in four patients (patients 1, 4, 5, and 6). Patient 2 complained of anterior chest pain on day 8 after the initiation of lactosucrose, and nitroglycerin was used once. After this episode, occult blood soon appeared, and he suffered from abdominal pain and bloody stool on the 6th day after the administration of lactosucrose had been stopped. Patient 7 had anemia due to bleeding from the colon and received a transfusion of packed red cells (total 600 ml) 2 weeks before the beginning of the study. Occult blood was also detected in patients 2, 3, 4, and 5, but the extent of bleeding did not change throughout the study period.

The erythrocyte sedimentation rate (ESR) and c-reactive protein (CRP) values were decreased or unchanged during the study period, except in patient 7. In patient 7, serum concentrations of total protein and albumin, white blood cell and red blood cell counts, and ESR and CRP values were increased, probably due to blood transfusion (data not shown). Administration of lactosucrose did not change other laboratory findings (data not shown).

Effect on lactosucrose on fecal microflora

Lactosucrose is indigestible and it therefore reaches the intestine. Lactosucrose has been reported to be selectively utilized by *Bifidobacterium*, but not by other pathogens.[13] The total fecal bacterial counts (log[counts/g wet feces]) were 10.6 ± 0.4 (mean ± SD, $n = 7$) on day 0, 10.6 ± 0.4 ($n = 7$) on day 7, 10.7 ± 0.5 ($n = 7$) on day 14, and 10.7 ± 0.3 ($n = 7$) on day 21. Thus, the total counts were not changed during the study period (Student's *t*-test). The frequency of detection of major bacteria and yeast and the mean ± SD values for positive samples are shown in Table 3. Before lactosucrose supplementation, *Bifidobacterium* was detected in three patients (patients 3, 4, and 6). Lactosucrose did not significantly increase the *Bifidobacterium* count in the positive feces during the study period (values shown in parentheses in Table 3), but after 2 weeks of lactosucrose administration, *Bifidobacterium* was found in the feces of all patients. Lactosucrose had a significant effect in inducing the growth of *Bifidobacterium* on day 14 ($P < 0.05$ by Fisher's exact probability test).

The population levels of fecal bacteria are shown in Table 4. The mean ± SD value for the relative level

Table 3. Effects of lactosucrose (*LS*) intake on bacterial count and frequency of detection

Bacterium	Positive samples/total number of patients (bacterial counts[a])			
	Before LS intake	During LS intake		After LS intake
	Day 0	Day 7	Day 14	Day 21
Enterobacteriaceae	6/7 (9.0 ± 0.5)	7/7 (9.3 ± 1.0)	7/7 (9.4 ± 0.6)	7/7 (9.2 ± 0.6)
Streptococcus	7/7 (9.1 ± 0.7)	7/7 (9.4 ± 0.7)	7/7 (9.4 ± 0.9)	7/7 (9.6 ± 0.6)
Staphylococcus	4/7 (3.3 ± 0.6)	3/7 (3.6 ± 0.7)	3/7 (3.8 ± 0.7)	4/7 (3.4 ± 0.5)
Yeast	5/7 (4.8 ± 0.4)	5/7 (6.5 ± 2.0)	4/7 (4.7 ± 0.6)	5/7 (5.1 ± 0.9)
Lactobacillus	4/7 (7.3 ± 3.0)	6/7 (8.0 ± 1.3)	6/7 (8.3 ± 1.3)	6/7 (8.4 ± 1.4)
Bifidobacterium	3/7 (9.4 ± 1.2)	5/7 (10.0 ± 0.7)	7/7[b] (9.6 ± 0.9)	5/7 (9.5 ± 1.0)
Eubacterium	6/7 (9.9 ± 0.3)	6/7 (9.6 ± 0.8)	7/7 (10.1 ± 0.5)	7/7 (10.0 ± 0.6)
Bacteroidaceae	6/7 (10.3 ± 0.4)	7/7 (10.0 ± 0.6)	7/7 (10.2 ± 0.5)	7/7 (10.2 ± 0.4)
Peptococcaceae	3/7 (9.5 ± 0.7)	5/7 (9.3 ± 0.4)	5/7 (8.8 ± 1.0)	5/7 (9.3 ± 0.3)
Clostridium perfringens	2/7 (7.5 ± 2.4)	4/7 (6.3 ± 1.9)	2/7 (6.4 ± 1.5)	5/7 (4.9 ± 1.5)
Clostridium (other)	4/7 (6.0 ± 2.2)	4/7 (6.4 ± 2.3)	4/7 (6.7 ± 2.4)	5/7 (5.0 ± 2.9)
Veillonella	1/7 (7.1)	1/7 (4.1)	0/7 n.d.	2/7 (7.3 ± 2.1)

[a] Values are means ± SD (log [numbers of bacterial counts/g wet faces]) for respective bacterium-positive feces
[b] Frequency of detection significantly increased compared to that before LS intake, by Fisher's exact probability test ($P < 0.05$)

Table 4. Effect of lactosucrose intake on the relative population level of fecal bacteria (%)

	Before LS intake	During LS intake		After LS intake
	Day 0	Day 7	Day 14	Day 21
Bifidobacterium	3.9 ± 7.2	15.8 ± 17.2*	12.6 ± 10.9*	8.9 ± 13.1
Eubacterium	18.9 ± 11.6	12.5 ± 9.3	27.0 ± 14.7	25.5 ± 19.3
Bacteroidaceae	46.9 ± 29.5	29.9 ± 18.4*	36.4 ± 17.0	34.2 ± 16.9
Aerobes	27.0 ± 34.6	38.1 ± 37.7	21.7 ± 15.0	27.8 ± 25.0
Other	3.4 ± 4.4	3.6 ± 3.9	2.4 ± 2.7	3.7 ± 3.4

* $P < 0.05$, Significant difference from values before LS intake by Wilcoxon signed-rank test
Values are means ± SD for seven samples

of each microbe was obtained from both bacteria-positive and -negative feces. Lactosucrose intake significantly increased the relative population level of *Bifidobacterium* on days 7 and 14 of administration ($P < 0.05$ by Wilcoxon signed-rank test). Seven days after lactosucrose was stopped, *Bifidobacterium* was not detected in the feces of patients 1 and 2, and the relative level decreased to the pre-administration level (Table 4). In contrast to *Bifidobacterium*, the relative levels of Bacteroidaceae were significantly decreased by lactosucrose on day 7 of administration ($P < 0.05$ by Wilcoxon signed-rank test) (Table 4).

Alterations of fecal metabolites with lactosucrose

The growth of *Bifidobacterium* with the administration of lactosucrose for 14 days decreased the fecal pH by 0.55 ± 0.36 (mean ± SD) in patients 1, 3, 4, 5, and 7 (Fig. 1A). The total content of short-chain fatty acids on days 7 and 21 after the beginning of lactosucrose administration was significantly higher ($P < 0.05$ by Wilcoxon signed-rank test) than that before administration (Fig. 1B). Of the short-chain fatty acids measured, acetic acid significantly increased with lactosucrose, from 2659 ± 1464 μg/g wet feces (mean ± SD, $n = 7$) to 3286 ± 1504 on day 7, to 3989 ± 1912 on day 14, and to 4965 ± 1613 on day 21 ($P < 0.05$ by Wilcoxon signed-rank test); butyric acid also increased, from 463 ± 597 μg/g wet feces (mean ± SD, $n = 7$) to 742 ± 748 on day 14 and to 982 ± 772 on day 21 ($P < 0.05$ by Wilcoxon signed-rank test). The content of the other organic acids was not significantly changed during the study period. The increase in acetic acid content mainly accounted for the significant increase in the values for the total content of short-chain fatty acids. *Bifidobacterium* was also detected in patients 2 and 6, but it did not lower fecal pH or increase the content of short-chain fatty acids (Fig. 1).

The effects of lactosucrose on fecal putrid products and urinary indican levels are shown in Fig. 2. Total putrid products, estimated by measuring indoles and phenols, were not significantly decreased with lac-

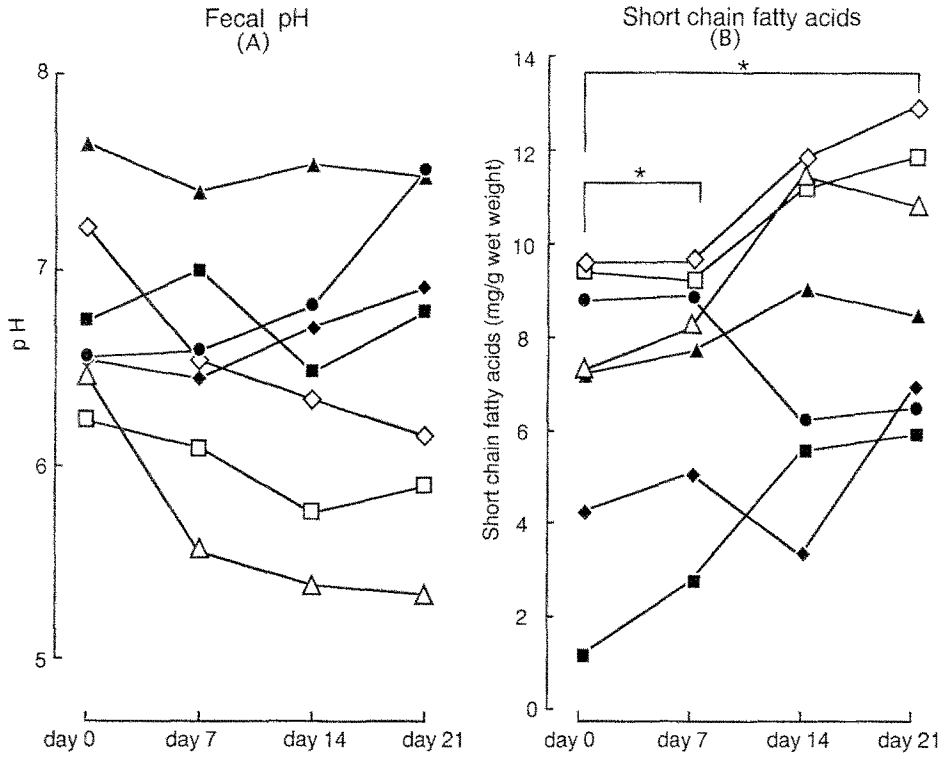

Fig. 1A,B. Effect of lactosucrose intake on **A** fecal pH and **B** content of short-chain fatty acids. Fecal pH and the content of short-chain fatty acids in feces from individual patients were analyzed as described in Materials and Methods. $^*P < 0.05$, significantly different from values on day 0 by Wilcoxon signed-rank test. *Solid squares*, case 1; *solid diamonds*, case 2; *open triangles*, case 3; *open diamonds*, case 4; *open squares*, case 5; *dots*, case 6; *solid triangles*, case 7

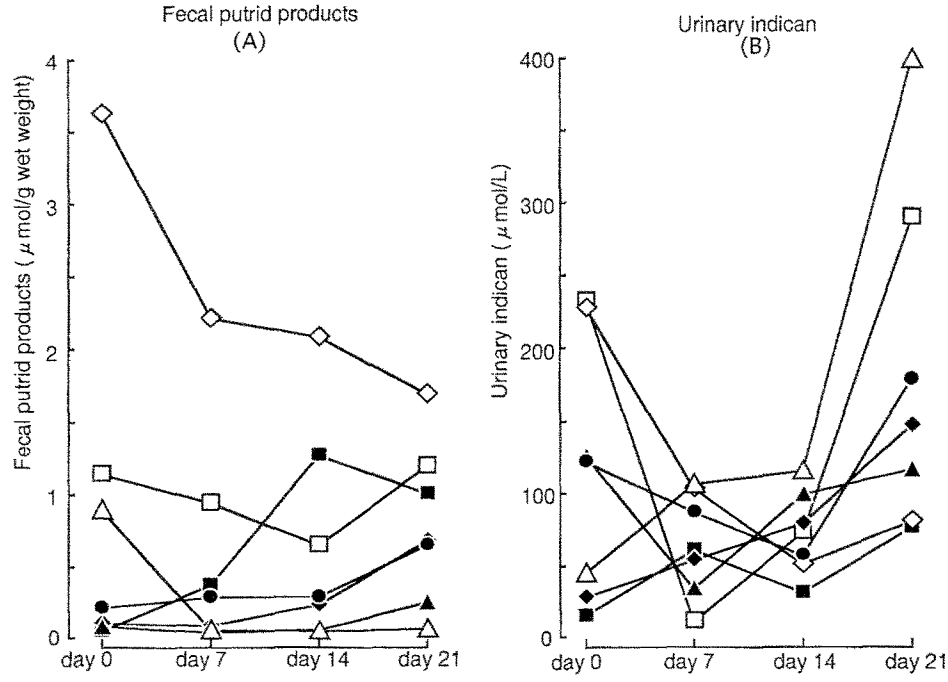

Fig. 2A,B. Effect of lactosucrose on **A** fecal putrid products and **B** the urinary secretion of indican. The values expressed for putrid products are total amounts of the fecal content of indole, skatole, phenol, *p*-cresol, and 4-ethylphenol. These putrid products were measured as described in Materials and Methods. *Symbols*, As in in Fig. 1 legend

tosucrose, but lactosucrose significantly decreased the levels of phenols, from 579 ± 1017 ng/g wet feces (mean ± SD, $n = 7$) on day 0 to 321 ± 617 on day 7 ($P < 0.05$ by Wilcoxon signed-rank test). The levels of these products in the intestine were also estimated by measuring urinary indican. Urinary indican was decreased in patients 4, 5, 6, and 7 (Fig. 2B), but the decrease was not significant.

Discussion

Lactosucrose is an indigestible oligosaccharide synthesized from sucrose and galactose by β-fructofuranosidase. Fujita et al.[14] examined its digestibility in vitro and suggested that more than 90% of lactosucrose reached the intestine. Lactosucrose is a good nutrient for *Bifidobacterium* and has been reported to selectively induce the growth of *Bifidobacterium* in healthy subjects,[13] in the elderly suffering from constipation,[20] and in patients with a resected colon.[21,22] *Bifidobacterium* metabolizes lactosucrose to produce short-chain fatty acids, which are not only a good energy source for enterocytes but also produce an acidic environment, preventing the growth of anaerobic bacteria. Iwagaki et al.[23] reported that preoperative administration of lactosucrose suppressed the growth of anaerobic bacteria, especially Bacteroidaceae, and decreased serum endotoxin levels.

The composition of adult human microflora is considered to be relatively stable. However, in patients with Crohn's disease or ulcerative colitis, anaerobic bacteria, including *Bifidobacterium*, Bacteroidaceae, *Enbacterium*, Peptococcacae, and *Lactobacillus*, have been reported to be markedly decreased.[3,5] Long-term administration of an elemental diet to patients with Crohn's disease has also been suggested to alter the composition of microflora and to specifically increase *Clostridium*.[11] However, studies using conventional culturing techniques have given inconsistent results. We carefully carried out bacterial examination immediately after collecting samples in anaerobic bags. At the same time, metabolic alterations in feces and urine were also examined. A shift in the relative proportions of the various strains has been suggest to cause changes in the metabolic properties of the bacteria or in the complex interaction between the microbes and the host. The alterations in the intestinal microflora result in increases in the production of indole and phenol derivatives. These volatile amino acid metabolites, which are used to detect metabolic alterations in the intestine, are known to be toxic to cells and to be involved in mutagenesis.[24–26] The frequency of detection of fecal phenols, especially *p*-cresol, was found to be higher in patients with ulcerative colitis than in healthy subjects.[12] Thus, functional alterations of the intestinal microflora must be considered when we provide nutritional support for patients with chronic inflammatory bowel disease.

In this study, we found that the administration of lactosucrose did not change total bacterial counts, but effectively induced the growth of *Bifidobacterium* in the feces of ulcerative colitis and Crohn's disease patients. In contrast, lactosucrose significantly decreased the relative population of Bacteroidaceae in these feces. In association with the growth of *Bifidobacterium*, bowel movements improved in four out of seven patients. The improvement of the intestinal environment, possibly induced by the growth of *Bifidobacterium*, was shown indirectly in terms of the measurement of fecal pH, the fecal content of short-chain fatty acids, and the levels of putrid products in the feces and urine. Lactosucrose significantly increased the content of short-chain fatty acids in the feces and significantly decreased the levels of phenols in the feces. The other values also were improved (but not significantly). Except for patient 2, the value of more than one parameter improved with the administration of lactosucrose. The administration of lactosucrose in this study did not appear to any cause of side effects. Since the present study involved the short-term administration of lactosucrose in a limited number of patients, our results do not definitively confirm the beneficial effects of lactosucrose in chronic inflammatory bowel disease. We did not have a control group that received other in digestible oligosaccharides, and other such agents may show different changes in the composition of the intestinal microflora. The clinical courses of the patients examined in this study were stable, and each patient received the same medication and diet during the study period. The composition of the adult human microflora is considered to be relatively stable under these conditions.[27,28] After the withdrawal of lactosucrose, the composition of the fecal microflora returned to that before the administration of lactosucrose (Tables 3 and 4), suggesting that lactosucrose may selectively stimulate *Bifidobacterium* growth and have a beneficial effect on patients with chronic inflammatory bowel disease. Further study of the long-term administration of lactosucrose to a large number of patients with chronic inflammatory disease will be an important next step.

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