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CLINICAL EFFECTS OF BIFIDOBACTERIA AND LACTOBACILLI

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SUMMARY

To clarify the rationale of administration of *Bifidobacterium* to humans, we evaluated the faecal microflora in different age groups from the view point of colonisation resistance. Our findings suggest that the number of *Bifidobacterium* is the potential marker of the stability of the human intestinal microflora. On the clinical effects of *Bifidobacterium,* administration of *B. breve* preparation (BBG-01; 10^9 cells/g) to the patients with *Campylobacter* enteritis (n=133) showed the enhancement of eradication of *Campylobacter jejuni* along with the recovery of normal flora. In the cases of infantile intractable diarrhoea primarily induced by antibiotics (n=15, mean 2.5 yr.), the stool frequency and appearance were dramatically improved within 3 to 7 days after administration of BBG-01 from chronic watery diarrhoea (mean 25 days), with normal flora predominating resident *Bifidobacterium* or administered *B. breve*. On the clinical effects of *Lactobacillus casei* Shirota strain, oral administration of *L. casei* preparation (BLP: 10^{10} cells/g) is useful for the prevention of the recurrence of superficial bladder cancer: the 50% recurrence free duration was prolonged significantly by BLP treatment (n=23, 350 days) to 1.8 times that in control group (n=25, 195 days). The suppressive effect of BLP on the specific urinary mutagenicity derived from the ingestion of cooked meat was demonstrated in 6 healthy non-smokers. BLP administration for 3 weeks resulted in the decrease of urinary mutagenicity (6-67%, average 47.5%) compared to before administration. The blood pressure-lowering effects of oral administration of extract from *L. casei* (LEx) on the systolic blood pressure (SBP) were demonstrated in spontaneously hypertensive rats (SHR), widely used as an animal model for hypertension. Oral doses of 1 to 10 mg/kg of LEx yielded a significant decrease of SBP in the SHRs, but had no effect on normotensive rats. Clinical application of LEx in patients with hypertension is under investigation.

INTRODUCTION

In 1907, *Metchnikoff* launched the theory that the intestinal microflora exerts important influences on health and longevity. Although controversial, his theory was responsible for much subsequent scientific research regarding the role of fermented and culture-containing dairy products in health. Research performed with germfree animals and the introduction of improved anaerobic

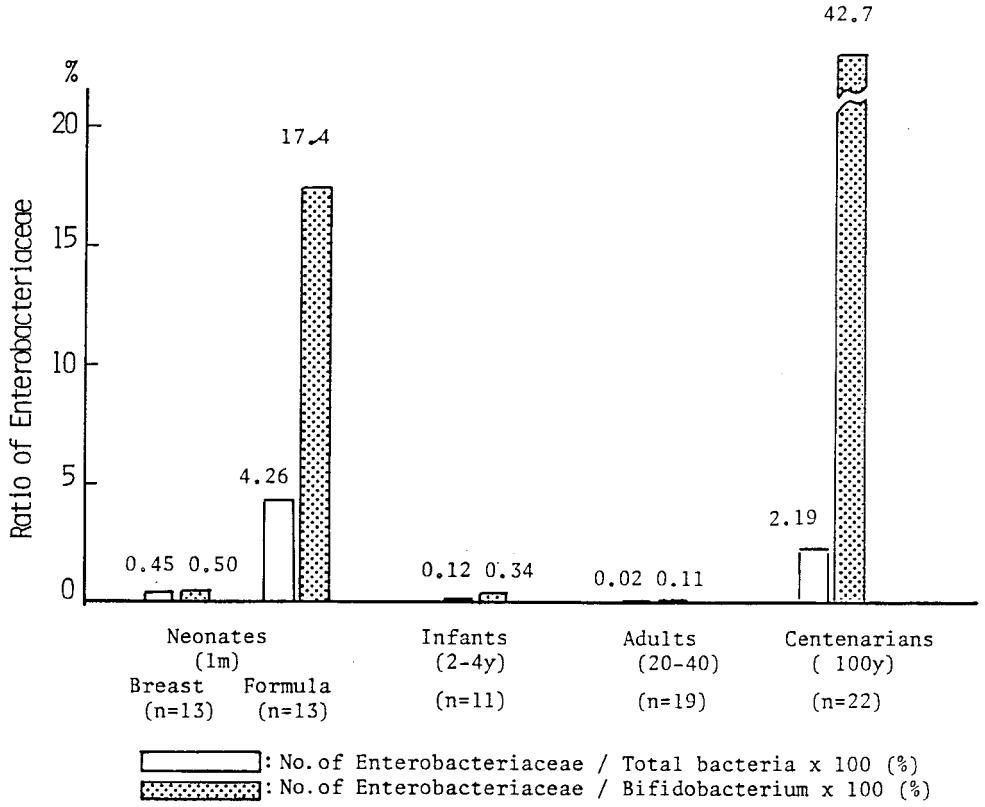

Figure 1: Colonisation resistance of human intestinal flora: the ratio of non-anaerobes to aerobes (from: *Mutai* and *Tanaka*, 1987).

culture techniques have been particularly useful in clarifying the significance of the interrelationships of diet and intestinal microflora in health and disease (*Mitsuoka*, 1982; *Finegold*, 1983; *Mallett*,1988). In spite of a rather large list of literature, the therapeutic effects of the preparations of bifidobacteria or lactobacilli as well as their culture-containing dairy products on human gastrointestinal disorders remain obscure or controversial. A criticism of most of the reports on the clinical effects of the administration of bifidobacteria or lactobacilli have been well summarised previously (*Conway*, 1989; *Renner*, 1991). The problems are:

1) not all the data reported in literature are based on well designed and controlled experiments and often lack statistical analysis, and

2) results obtained have to be evaluated based on the understanding of the specificity and stability of gastrointestinal ecosystem.

In view of this, the aim of this paper is to review the recent clinical effects of the administration of our Yakult strains of *Bifidobacterium breve* and *Lactobacillus casei* Shirota or their fermented dairy products in humans in a strictly controlled study.

RATIONALE OF ADMINISTRATION OF BIFIDOBACTERIA IN HUMANS

It has been recognised that breast-feeding protects infants from diarrhoea and a variety of respiratory infections which are major cause of infantile morbidity and mortality. Extensive studies have shown the significance of anti-infectious factors in breast milk, notably the maternal immune factors and the Bifidus factors. Much efforts have been made for the propagation of *Bifidobacterium* in bottle-fed infants either by the direct implantation of *Bifidobacterium* or adding Bifidus-growth promoting factors. In addition, it is well known that the normal intestinal flora is stable and prevents the colonisation of a number of environmental microbes including pathogenic bacteria. The protection against colonisation is involved in the natural resistance of humans to intestinal infections. *Van der Waaij* and colleagues (1972) proposed the concept of colonisation resistance and emphasised an important role for anaerobes in the maintenance of colonisation resistance in the intestines. In general, the ratio of non-anaerobes to anaerobes seems to be an indicator of the potential stability of the intestinal flora. Figure 1 shows the comparative values of the human intestinal flora in the different age groups according to our results on the faecal microflora of a group of healthy humans, consisting of 13 breast-fed and 13 formula-fed infants, 11 children, 19 adults, and 22 Okinawan centenarians, the world's reliable region on the geography of longevity (Table 1). The number of Enterobacteriaceae and total bacteria were used as the representatives of non-anaerobes and anaerobes, respectively. In addition, the number of Bifidobacteria, as the representatives of the most useful bacteria in the human intestine, was also used instead of total bacterial count. In the formula-fed infants and centenarians, the ratio of non-anaerobes to anaerobes was a factor of 10 to 100 higher than that of breast-fed infants, weanlings, and adults. Furthermore, when using the number of Bifdobacteria instead of total bacteria, these differences were more striking, suggesting that *Bifidobacterium* plays an important role in the maintenance of colonisation resistance. It is of interest that formula-fed infants and the aged are more susceptible to various infections than the other age groups.

EFFECT OF *BIFIDOBACTERIUM BREVE* ON INFANTILE DIARRHOEA

Diarrhoeal diseases are the major cause of morbidity and mortality in infants and young children, especially in developing countries. *Shigella*, enteropathogenic *Escherichia coli*, enterotoxigenic *E. coli*, *Campylobacter* and rotavirus are the most important aetiological agents (*Chen*, 1978). The gastroenteris due to *Campylobacter* and *Salmonella* are the most common cause of acute infectious diarrhoea in developed nations (*Chen*, 1978). In addition, the administration of broad-spectrum antibiotics often results in antibiotic-associated diarrhoea, the so called non-specific watery diarrhoea syndrome, and pseudomembranous enterocolitis due to *Clostridium difficile* (*Bartlett*, 1983).

Table 1: Composition of faecal microflora of healthy human in different age groups (from: *Mutai* and *Tanaka*, 1987)

Age group	Total bacteria	Bacteroidaceae	*Bifidobacterium*	*Clostridium* (L+)	Enterobac-teriaceae	*Enterococcus*	*Lactobacillus*	*Staphylococcus*
I: neonates (1 m) breast-fed (n=13)	10.77±0.33	9.31±0.24 (100)	10.72±0.38 (100)	7.25±2.58 (16)	8.42±0.91 (100)	6.78±1.73 (100)	7.05±1.36 (64)	6.15±1.58 (93)
II: neonates (1 m) formula-fed (n=13)	10.64±0.39	9.84±0.60 (100)	10.03±0.55 (85)	6.87±2.65 (16)	9.27±0.72 (100)	8.35±1.33 (100)	6.77±1.89 (77)	5.70±1.12 (100)
III: infants (2-4 y; n=11)	10.67±0.18	10.03±0.31 (100)	10.22±0.26 (100)	4.93±1.24 (91)	7.75±0.52 (100)	7.85±0.94 (100)	5.28±1.65 (100)	3.69±0.84 (100)
IV: adults (20-40 y; n=19)	10.98±0.28	10.72±0.32 (100)	10.30±0.32 (100)	4.17±1.56 (84)	7.33±0.78 (100)	6.67±1.11 (100)	6.24±1.71 (90)	4.10±0.92 (70)
V: centenarians (>100 y; n=22)	10.35±0.25	9.95±0.43 (100)	9.06±0.57 (86)	6.01±2.20 (91)	8.69±0.89 (100)	7.68±1.44 (100)	6.30±1.58 (91)	5.52±1.88 (77)
Significance								
I : II		**	**		*	*		
I : III		***	**		*		*	***
I : IV		***	**		***			***
I : V	***	***	***					
II : III					***			***
II : IV	**	***			***	***		**
II : V	*		***					
III : IV	**	***				**		
III : V	***		***		**			**
IV : V	***	***	***	**	***	*		*

Mean ± SD of log bacterial counts per gram faeces (%; the rate of occurrence).
Significant difference: *p<0.05; **p<0.01; ***p<0.001

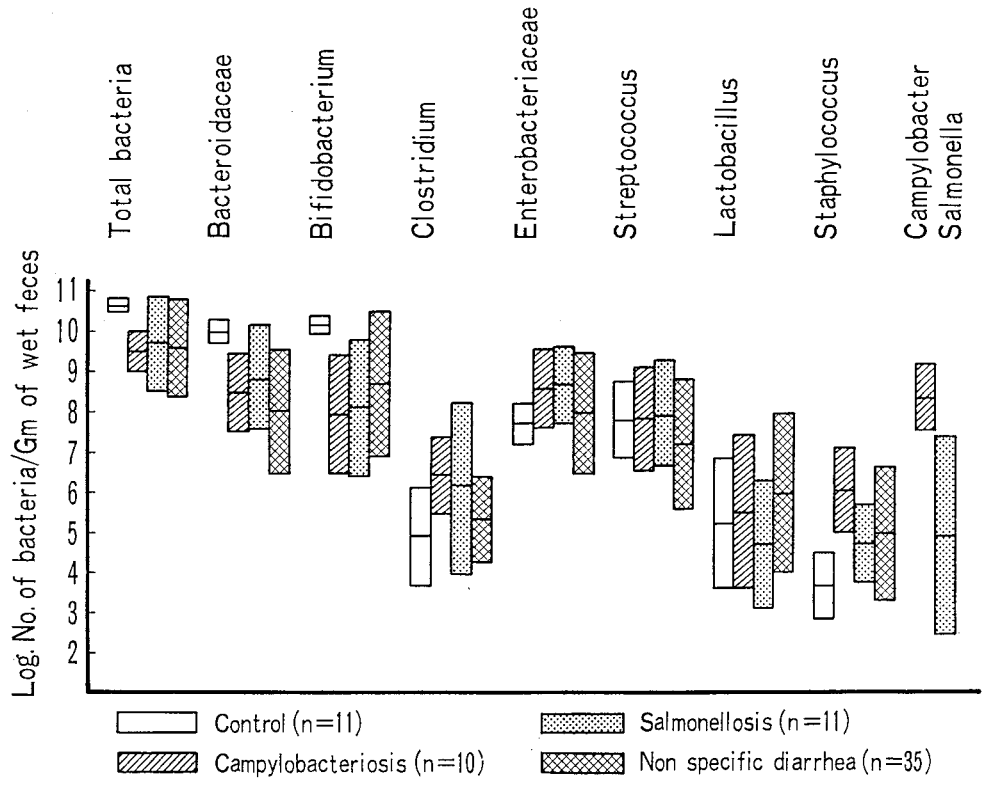

Figure 2: Faecal microflora of infants with diarrhoeal disease (from: *Tanaka* et al., 1989).

THE FAECAL MICROFLORA OF PATIENTS WITH ACUTE GASTROINTESTINAL INFECTIONS

In our institute, a total of 56 outpatients with acute diarrhoea at a modern University hospital in Tokyo were studied (*Tanaka* et al, 1990). Briefly, all faecal specimens were obtained by taking rectal swabs, and were transported to our institute in an anaerobic transport-medium. Medium preparation, dilution and inoculation were carried out anaerobically according to the modified VPI anaerobic roll tube methods. MVLG-KV (modified VL-G, MVLG, containing 80 µg/ml kanamycin and 1.0 µg/ml vancomycin, MPN, CW (Nissui, Tokyo) were used for counting total bacteria, Bacteroidaceae, *Bifidobacterium* and lecithinase positive *Clostridium,* respectively. The administered *Bifidobacterium breve* Yakult strain was enumerated using MPN *Bifidobacterium* selective medium containing streptomycin (3000 µg/ml) and neomycin (100 µg/ml). The numbers of Enterobacteriaceae, *Enterococcus, Lactobacillus* and *Staphylococcus* were determined using the selective medium of either DHL (Nissui), KMN or Staphylo No. 110 (Nissui). For the isolation of fungi, Candida GS medium (Eiken, Tokyo) was used. For the detection of enteropathogens, *Shigella, Salmonella* and *Yersinia* were examined using SS medium(Nissui), *Vibrio parahaemolyticus, Vibrio cholera* using TCBS medium (Eiken) and *Campylobacter* using Skillow's medium (Nissui). At

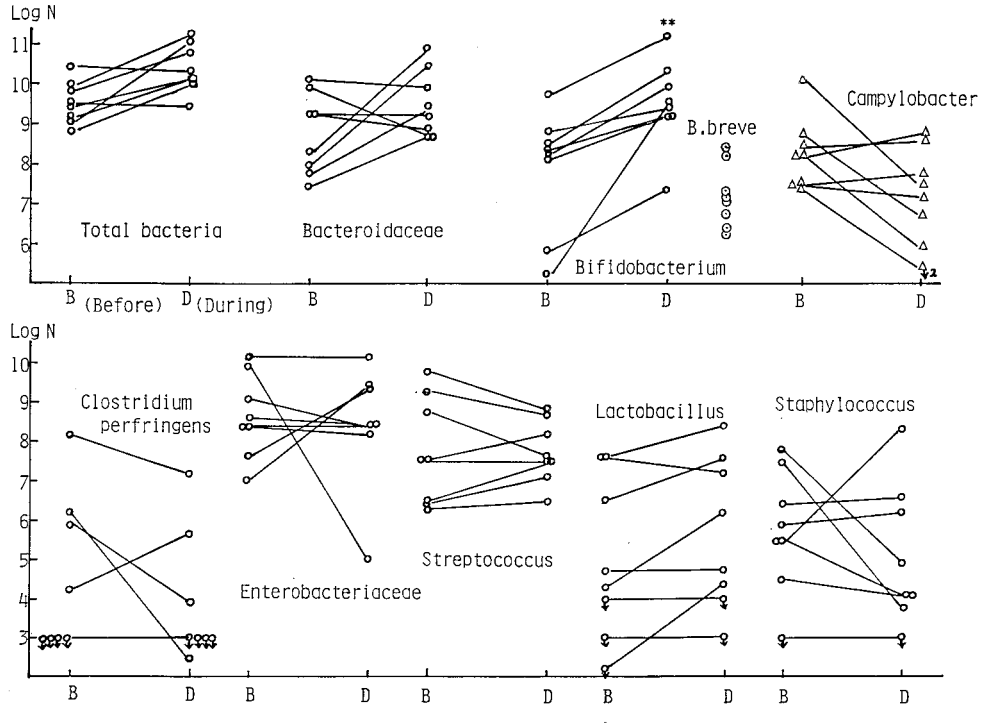

Figure 3: Effect of bacteriotherapy on the faecal flora of *Campylobacter* enteritis. Bacteriotherapy: administration of BBG-01 (*B. breve* preparation, 10^9 per day for 1 week; from: *Tanaka* et al., 1990).
**p<0.01 (Student's paired t-test)

least five strains of *Clostridium difficile* and enterotoxigenic *E. coli* were examined from each specimen using CCFA medium (Nissui) and DHL, respectively. The presence of toxin production was examined for each bacterium. Faecal cytotoxin and enterotoxin produced by *C. difficile* were determined by means of cytopathogenic effects (CPE) on HeLa cells and CPE neutralisation by anti-*Clostridium sorderii* antibody, kindly provided by Prof. Nakamura, School of Medicine, Kanazawa University. Enterotoxin was determined by means of a reversed passive latex agglutination kit (Denka Biological Laboratories, Tokyo). Heatlabile (LT) and heat stable toxin (ST) of enterotoxigenic *E. coli* were determined by a commercially available kit (Denka) and by intragastric administration in infant mice.

In the outpatients studied, the most commonly found enteropathogens were *Salmonella* (11 cases) and *Campylobacter* (10 cases) next to non-specific diarrhoea (35 cases). In the faecal microflora at the acute stage of diarrhoea, the number of anaerobes; total bacteria, Bacteroidaceae and *Bifidobacterium* were significantly reduced along with an increase of Enterobacteriaceae, *Staphylococcus,* and *Clostridium* (lecithinase positive), regardless the difference of the causative agents when compared to age-matched healthy infants (Figure 2).

EFFECT OF A *BIFIDOBACTERIUM BREVE* PREPARATION ON *CAMPYLOBACTER* ENTERITIS

Since it is shown that *Campylobacter* enteritis is the most common gastrointestinal infection, the effect of administration of a *B. breve* preparation, BBG-01 containing 3 x 10^9 cells/g, on the faecal flora of 8 patients with *Campylobacter* enteritis was investigated at first (Figure 3). During bacteriotherapy, the administered *B. breve* was recovered at a level of 10^6 to 10^8/g. In addition, the increase in number of total bacteria, Bacteroidaceae and *Bifidobacterium* was observed along with a decrease of *Campylobacter*. These results suggest that the administration of *B. breve* plays an important role in restoring the normal intestinal flora and thus shortens the time needed to eradicate *Campylobacter*.

In order to clarify in more detail the clinical effects of the *B. breve* preparation on *Campylobacter* enteritis, a total of 133 patients, aged 6 month to 15 years who had diarrhoea with *Campylobacter jejuni* alone, were used and randomly divided into three groups at the first visit to the hospital or clinic (*Tojyo* et al., 1987). Patients with mixed cultures of known gastrointestinal pathogens other than *C. jejuni* were excluded from the study. Briefly, the patients in group I (n=36) were treated with erythromycin (EM) at a concentration of 30-50 mg/kg daily in three divided doses for 7 days with additional antidiarrhoeal medication (Albumini Tannas and Alumini Silicas Naturalis). In group II (n=60), patients were given *B. breve* preparation (BBG-01) daily in three divided doses until *C. jejuni* was eradicated from the stool specimens. The additional antidiarrhoeal medication was also given in a similar manner as in group I. In group III (n=37), considered as the control group, patients were given only antidiarrhoeal medication until the symptoms were disappeared. In all patients, stool cultures were performed at least once a week until two consecutive cultures had become negative. Before bacteriotherapy, there were no differences between the three groups with regard to age, sex, maximum number of loose stools per day, blood in stool and fever (Table 2). The mean duration of diarrhoea was 2.7 days in

Table 2: Characteristics of patients with *Campylobacter* enteritis before treatment (from: *Tojyo* et al., 1987)

		I. Erythromycin n=40	II. BBG-01 n=60	III. Control n=37
Age:	< 2 yr.:	10	14	8
	2 - 5 yr.:	20	30	16
	> 5 yr.:	10	16	13
Sex (M/F):		22/28	34/26	24/13
Maximum number of loose stool/day (mean):		5.4	5.8	5.5
Blood in stool (%):		45	42	38
Fever (%):		50	57	51

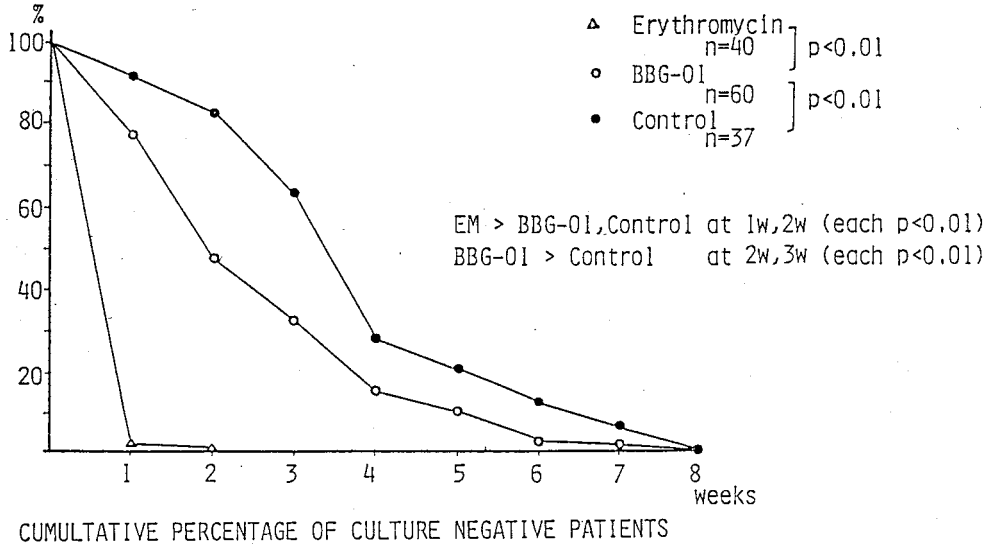

Figure 4: Effect of BBG-01 (*B. breve* preparation) on the eradication of *Campylobacter* (from: *Tojyo* et al., 1987).

the EM group, 3.2 days in the BBG-01 group and 2.7 days in the control group. The differences between these three groups were not statistically significant. On the effects of eradication of *Campylobacter jejuni*, as shown in Figure 4, EM is the most effective (EM>BBG-01, control, $p<0.01$), as was expected. However, BBG-01 is more effective than the control group (BBG-01>control, $p<0.01$). In addition, at weeks 2 and 3, the differences in the numbers of patients with a positive culture were also significant ($p<0.01$) between BBG-01 and control. In our study, EM did not alter the natural course of the disease in patients treated 7 days after the onset of symptoms, as has been reported by *Anders* and co-workers (1982). It was also demonstrated that the course of *Campylobacter* enteritis is self-limiting in normal children. Faecal carriage of *C. jejuni* persisted for up to 8 weeks if patients receiving no treatment and may be a source of contamination of the environment.

In summary, the administration of a *B. breve* preparation (BBG-01) was effective in eradicating *C. jejuni* and in restoring the normal intestinal flora. However, there were no differences in the duration of diarrhoea among three treatment groups (EM, BBG-01 and control antidiarrhoeal medication), regardless the different effects on the eradication of *C. jejuni*. This epidemiological result suggests that *B. breve* yoghurt may be useful for prophylaxis of infectious diarrhoea rather than for therapeutic use.

EFFECTS OF A *BIFIDOBACTERIUM BREVE* PREPARATION (BBG-01) ON ANTIBIOTIC-ASSOCIATED INTRACTABLE DIARRHOEA

A number of studies demonstrated that the suppression of the normal in-

Table 3: Summary of clinical responses of infants with intractable diarrhoea (from: *Hotta* et al., 1987)

Patient No.	Age	Sex	Underlying disease	Symptoms	Antibiotics	Bacteriotherapy	Duration of diarrhoea (days) before treatment	Duration of diarrhoea (days) after treatment
1	2y	F	periodic granulocytopenia	furuncle	CCL,CET,GM,PIPC	BLG-B	7	7
2	2y,10m	M		sepsis	KM,GM,ABPC,CBPC, CEZ,CMZ,CTX	BLG-B MTTMIL	30	14
3	1y	M	Kawasaki disease	bronchitis	CEX	MILMIL	35	7
4	1y	M		sepsis?	LMOX,CET	BBG-01	5	7
5	1y,8m	F	nephrosis	salmonellosis	ABPC,ST,KM,FOM	BLG-B	35	8
6	1m	M		bronchitis	ABPC,AMPC	BBG-01	25	7
7	15y	M	chronic nephritis	peritonitis	TOB,CET	BBG-01	11	6
8	1y,3m	M	Hirshsprung	sepsis?	TOB,CBPC,CTX,FOM GM,CAZ	BBG-01	10	3
9	1m	F	milk allergy	sepsis?	ABPC,CEZ,KM	BBG-01	25	7
10	3y	M	hemophilia B	sepsis?	CTX,CLDM,TOB	BBG-01	9	4
11	6y	M	hemophilia B	UTI	CTX,CXM	BLG-B	70	10
12	1m	M		sepsis?	ABPC,LMOX	BBG-01	7	4
13	3m	M	ventricular septal defect	sepsis	CTX,MCIPC,ABPC, LMOX,PIPC,CP	BBG-01	3-	10
14	4y,6m	F	Reye syndrome	broncho-pneumonia pulm. edema	ABPC,PIPC,CET,GM	BBG-01	40	7
15	3m	M		sepsis?	CMZ	BBG-01	40	4

BLG-B : Combined preparation of *Bifidobacterium breve* (10^9/g) and *Lactobacillus casei* (10^{10}/g).
BBG-01 : Preparation of *Bifidobacterium breve* (10^9/g).
MILMIL : Yoghurt containing 10^{10} of viable *Bifidobacterium breve*, *Bifidobacterium bifidum*, and 10^9 of *Lactobacillus acidophilus* per 100 ml.

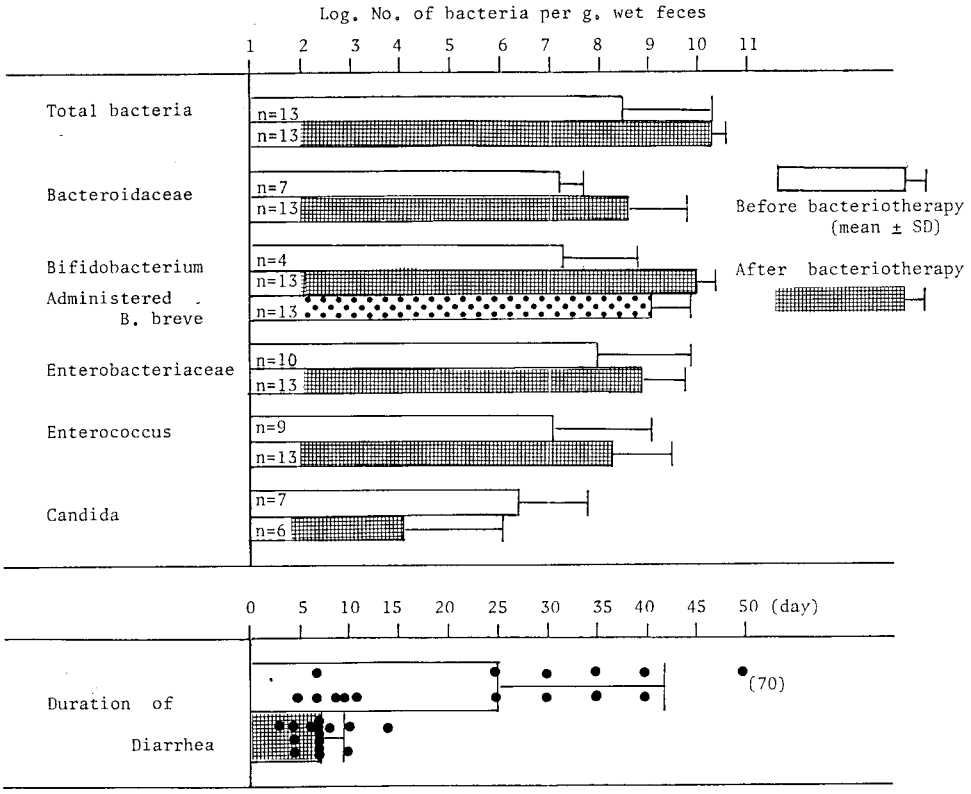

Figure 5: Effects of bacteriotherapy using *Bifidobacterium* on infantile intractable diarrhoea (from: *Hotta* et al., 1987).

testinal microflora leads to undesirable effects. It is well known, for example, that antibiotic therapy increases the risk of susceptibility to infections, bleeding problems and the selection of antibiotic-resistant strains. With regard to intestinal infections, the best known is pseudomembranous enterocolitis due to *Clostridium difficile.* Intractable diarrhoea in infancy is generally characterised by malnutrition, unresponsiveness to conservative therapy, and a high mortality. The most common definition of intractable diarrhoea in paediatric medicine was proposed by *Avery* et al. (1968):

1) diarrhoea continues for longer than two weeks,

2) occurs within three months after birth,

3) faecal cultures performed more than three times show no evidence of causative agents for the diarrhoea.

As for therapy, it is now recognised that administration of parenteral nutrients is the current basis for successful management of these infants.

We investigated the effects of the administration of the *B. breve* preparation (BBG-01) or commercially available *Bifidobacterium* yoghurt on infantile protracted diarrhoea primarily induced by antibiotic treatment (*Hoita* et al., 1987). The clinical features of the 15 patients are listed in Table 3. These patients (11 boys and 4 girls), ranging in age from 1 month to 15 years (mean 2.5 yr.), received antibiotic therapy for the treatment of extra-intestinal complaints such as septicaemia and respira-

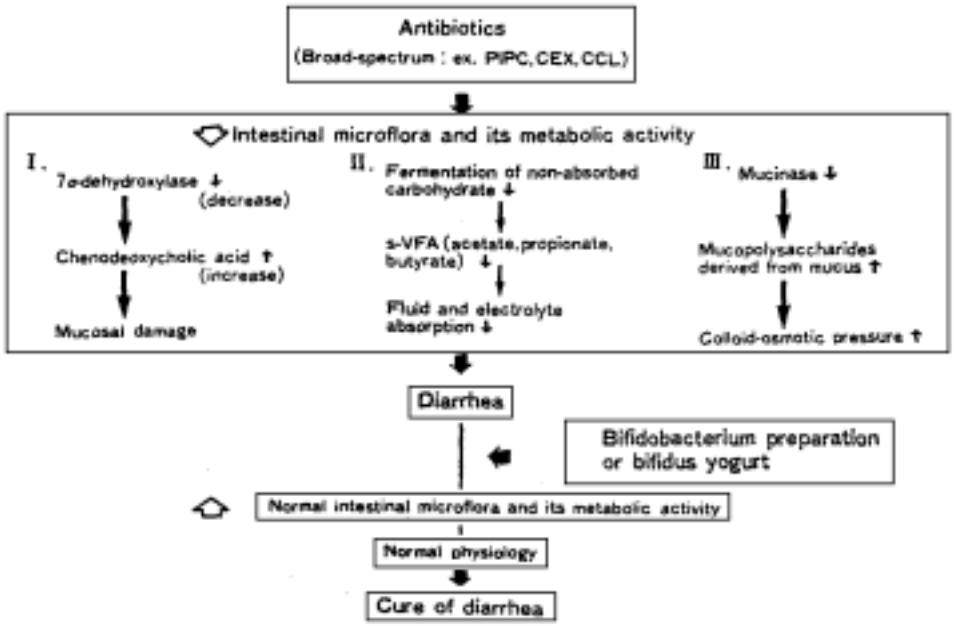

Figure 6: Some possible causes of antibiotic associated diarrhoea and cure mechanisms of *Bifidobacterium* therapy on infantile intractable diarrhoea (from: *Hotta* et al., 1987).

tory tract infections. The most frequently found infectious diseases in these patients were septicaemia (2 cases) and suspected septicaemia (6 cases), and respiratory infections (4 cases). Peritonitis, furuncles and *Salmonella* enteritis were found in one case each. The duration of diarrhoea prior to the onset of bacteriotherapy had an average of 25.3 days (ranging from 5-70 days) with deterioration of the general condition. In addition, conservative therapy (diet control, hyper-alimentation and chemotherapy) could not cure the diarrhoea. The antibiotics used included cephems, penicillins and aminoglycosides. Of the 15 patients, 10 patients received BBG-01; three combined preparations, BLG-B (*B. breve* at 10^9/g and *L. casei* at 10^{10}/g); the remaining two received either a combination of BLGB and Bifidus yoghurt (MILMIL) or Bifidus yoghurt alone containing 10^{10} cells of *B. breve*, *B. bifidum* and 10^9 of *L. acidophilus* per 100 ml. The intestinal microflora could be examined in 13 of the 15 patients. In most cases, an abnormal faecal flora was observed before bacteriotherapy; *Candida*, *Enterococcus* or Enterobacteriaceae often predominated with a marked decrease of anaerobes. *Bifidobacterium* was detected in only 4 of the 13 patients at 10^6/g wet faeces. During the disease, we could not detect any pathogens or toxins responsible for diarrhoea, such as *Clostridium difficile*. In all patients, the stool frequency and appearance dramatically improved within 1 week after oral administration of the *B. breve* preparation (Figure 5). After the diarrhoea was cured, the intestinal microflora of all the 13 patients studied normalised with a predominance of *Bifidobacterium* or administered *B. breve* at the level of 10^9 to 10^{10}/g. Figure 6 shows the possible mechanisms involved in the onset of antibiotic associated diarrhoea and in the recovery from diarrhoea with *Bifidobacterium*

Table 4: Characteristics of patients with superficial bladder cancer (from: *Aso* and *Akaza*, 1992)

		LC	Control	χ^2 test
Sex:	Male:	22	20	NS
	Female:	1	5	
Age (yr.):	<49:	0	2	NS
	50-59:	9	9	
	60-69:	6	8	
	>70:	8	6	
Stage:	pTa:	8	16	NS
	pTi:	14	8	
	pTx:	1	1	
Grade:	G1:	11	10	NS
	G2	12	15	
Papillary:		20	23	NS
Nonpapillary:		3	2	
Primary:		6	10	NS
Recurrent:		17	15	
Number:	Single:	5	4	NS
	Multiple:	18	19	
Size:	<1 cm:	17	17	NS
	1-3 cm:	6	7	
	>5 cm:	0	1	

bacteriotherapy. It is well known that the intestinal microflora has various metabolic activities that are essential for maintaining the normal physiological conditions of the host. Short-chain fatty acids (SCFA); acetic, propionic, and n-butyric acids are the major end products from unabsorbed carbohydrates of anaerobic microbial fermentation in the colon. It is reported that SCFA are rapidly absorbed and enhance the absorption of Na^+ and H_2O. Therefore, it can be said that the inhibition of anaerobes by antibiotic therapy probably reduces the luminal SCFA, resulting in malabsorption of H_2O and thus inducing diarrhoea. The administration of broad-spectrum antibiotics also did result in the decrease of bacterial 7α-dehydroxylation, which indicates inhibition of the conversion of primary bile acids to secondary bile acids. Thus, the increased concentration of primary bile acids, especially chenodeoxycholic acid may induce bile acid diarrhoea due to its detergent effects, causing injury to intestinal epithelial cells. Another possible explanation is the inhibition of bacterial degradation of mucopolysaccharides (mucin) secreted in the large intestine. The undegraded mucopolysaccharides may induce osmotic diarrhoea due to colloid-pressure induced fluid accumulation. This is suggested to be the pos-

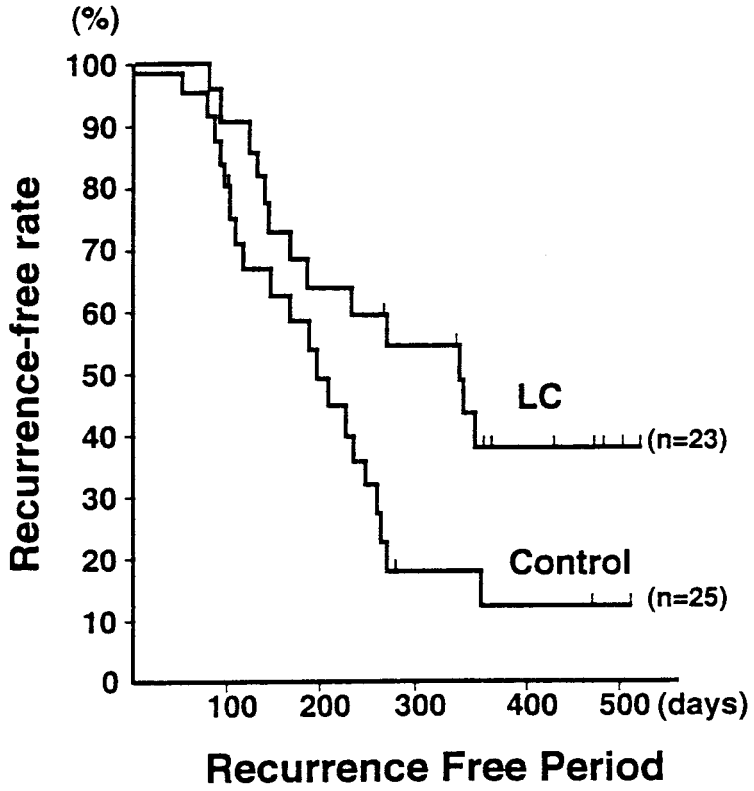

Figure 7: Effects of BLP (*L. casei* preparation, 10^{10} cells/g) on recurrence-free duration of superficial bladder cancer. The 50% recurrence-free intervals were 350 and 195 days for BLP and controls, respectively (Kaplan-Meier methods, *$p<0.05$; from: *Aso* and *Akaza*, a992)).

sible cause of caecal enlargement in germ-free rodents. In summary, malabsorption of water and electrolytes, probably induced by antibiotic therapy, may be corrected through normal metabolic activities such as SCFA production, bile acid metabolism and utilisation of mucin, resulting in a cure of diarrhoea. In summary, we stress the importance of the normal intestinal microflora, which plays an important role in the maintenance of normal physiology in the intestinal tract.

EFFECTS OF THE ADMINISTRATION OF *LACTOBACILLUS CASEI* ON HUMAN HEALTH

The beneficial role of *Lactobacillus* in the gastrointestinal tract has been one of the most controversial subjects within the area of gut microbial ecology (*Conway*, 1989). In the past decade, epidemiological and experimental studies suggested that there is an association between colon or breast cancer mortality and the so-called Western eating habit of consuming large amounts of animal protein and fat (*Wynder*, 1975, 1986). In this context, the more recent renewed interest in the nutritional significance of fermented dairy products has been fo-

cused on the possible preventive roles in colon and breast cancer through the improvement and stimulation of the microbial metabolic activity and immune system in the intestinal tract. In this section, the more recent evidences of the clinical effects of *Lactobacillus casei* on superficial bladder cancer, urinary mutagenicity and hypertension in humans will be reviewed.

Effect of *L. casei* on superficial bladder cancer

The prophylactic effect of a *Lactobacillus casei* preparation on the recurrence of superficial bladder cancer was demonstrated by *Aso* and *Akaza* (1992). A high recurrence rate as well as adverse side effects of chemotherapy for superficial bladder cancer have been of great concern to urologists. A total of 58 patients with superficial bladder cancer enrolled in this study at 19 institutions, in order to study the preventive effects of *L. casei* preparation (BLP) on the recurrence after transurethral resection of the bladder tumour (TUR-Bt). The patients were randomised into a BLP-treated group (n=23) and a control group (n=25). In the BLP-treated group, patients received daily 3 x 10^{10} viable cells of *L. casei* for about one year until the completion of the follow-up period or until tumour recurrence. Control cases received no medication or placebo for BLP. During the study period, none of the patients received any other medication, including chemotherapeutic drugs, antibiotics, etc., that might affect the effects of BLP. Throughout the study, patients were examined by routine laboratory tests, including haematological examinations, blood chemistry and urinalysis. To detect the tumour recurrence, endoscopy was performed every three months after enrolment. Cytological examination of the urine and bladder biopsies were performed if necessary. The disease-free duration and recurrence-free rate were determined by the Kaplan-Meier method. There were no significant differences in patients characteristics between two groups (Table 4). As shown in Figure 7, the comparison of the disease-free duration between two groups revealed that the 50% recurrence-free interval in the BLP group was 350 days, which was 1.8 times that in the control group (195 days). In summary, the authors suggested that BLP has the potentiality to posses comparable efficacy in the prevention of the recurrence of the bladder tumour to that obtained with intravesical instillation of some chemotherapeutic agents. In addition, BLP has more advantages over the intravesical instillation, being less adverse side effects and the fact that it can easily be administered by oral route.

Effects of BLP on urinary mutagenicity

Many kinds of heterocyclic amines are formed in cooked or grilled meat and fish. These heterocyclic amines have been shown to be carcinogens in humans (*Wakabayashi* et al., 1992). Therefore, many investigators are now focusing on their potential cancer risks as dietary carcinogens in humans. More recently, a suppressive effect of a BLP on the specific urinary mutagenicity derived from the ingestion of cooked meat, was demonstrated by *Hayatsu* et al. (1993) using the Ames test. Briefly, administration of BLP was started immediately after the first confinement period and was continued for 3 weeks. During the periods of the first confinement "Before control", and the second confinement indicated "After *L. casei*", a total of 6 subjects aged 28 to 37 years were offered the ordinary Japanese meal with 10 gram of cooked beef which was heated on the pan at 170°C to 190°C for

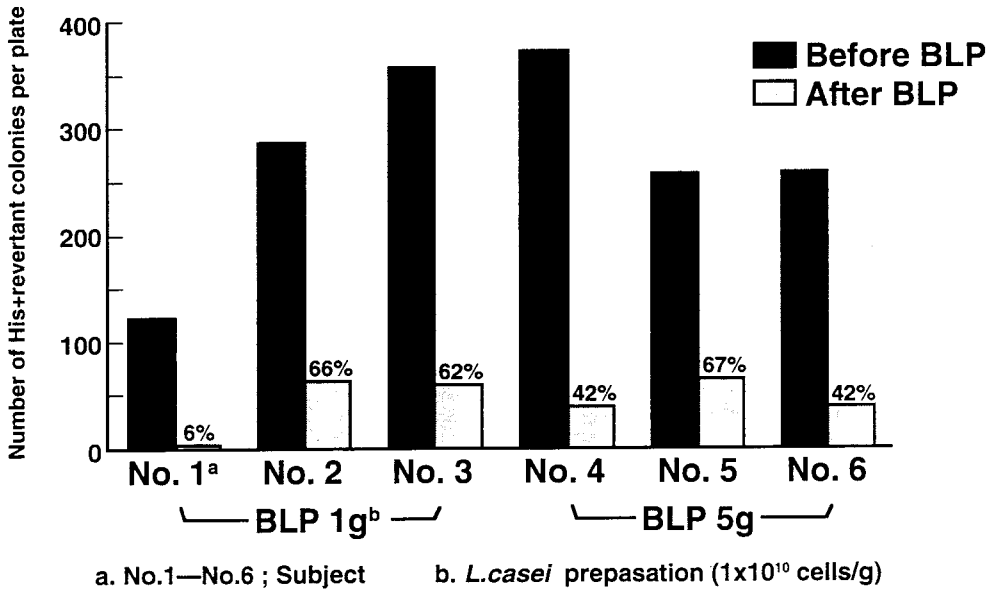

Figure 8: Effect of BLP (*L. casei* preparation) on beef meat-derived urinary mutagenicitiy (from: *Hayatsu* and *Hayatsu*, 1993).

10 min. The urinary mutagenicity was determined according to the Ames *Salmonella* test using *Salmonella typhimurium* TA98 with S9 mix. After BLP administration, the number of revertants decreased from 67 to 6%, compared to before BLP administration (Figure 8). There was a no difference in the suppressive effect of the BLP doses of 1 g or 5 g. The effective mechanisms are now under investigation in our institute, including the possibility of increased degradation of mutagens by the modified intestinal microflora as well as the mutagen binding by administered *L. casei.* In summary, the authors suggested that a decreased mutagen level in the urine by BLP administration may reflect a decrease in systemic mutagen exposure and may reduce cancer risks.

Effects of *L. casei* on hypertension

In our institute, the blood pressure-lowering effect of oral administration of the extract of autologous cell lysates from *L. casei* (LEx) on the systolic blood pressure (SBP) has been demonstrated in spontaneously hypertensive rats (SHR), a widely used animal model for hypertension. The clinical effects of LEx in patients with hypertension are now under investigation (*Hata* et al., personal communication). In brief, a total of 28 patients under treatment with anti-hypertensive drugs were randomised into two groups; given LEx (n=14) and a control group (n=14), fed dextrin as placebo. There were no differences in the clinical characteristics between the two groups. The LEx was administered orally at a dose of 800 mg per day for 2 months. The delta systolic blood pressure in patients given LEx was significantly lower than in the control group ($p<0.05$; data not shown). Antihypertensive compounds were purified from an extract of autrogous *L. casei* cell wall lysates (*Sawada* et al., 1990). The most effective compounds were polysaccharide-glycopeptide complexes, named SG-i, with a molecular

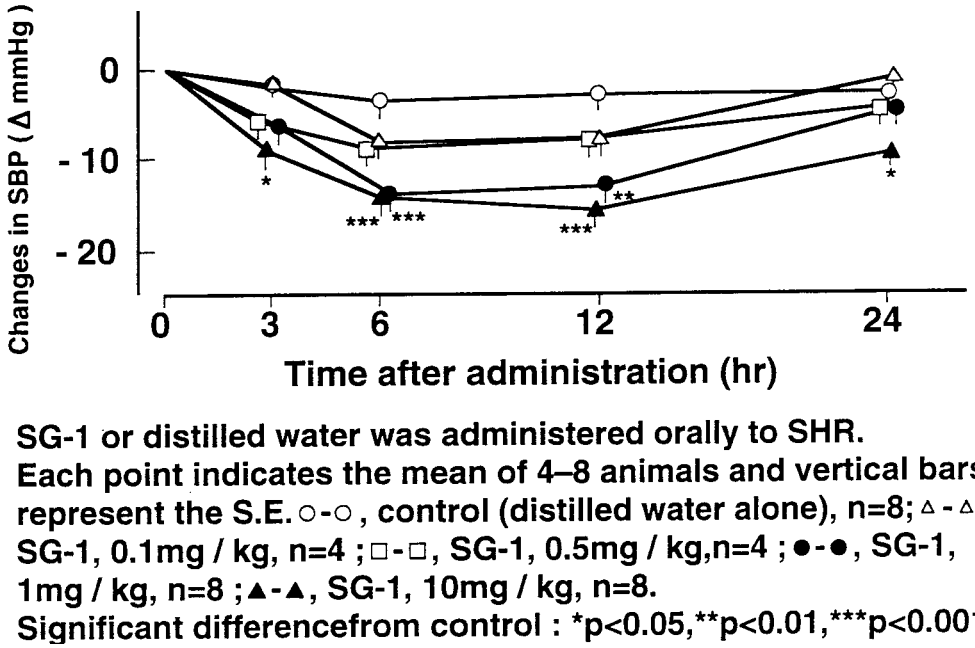

Figure 9: Effects of a single oral administration of SG-1 on the SBP of RHR (from: *Sawada* et al., 1990).

weight of about 180,000. The polysaccharide moiety of the complexes consisted of glucose, rhamnose, and galactose, whereas the glycopeptide moiety consisted of N-acetylglucosamine, N-acetylmuramic acid, asparagine, glutamine, alanine, and lysine. A significant decrease of systolic blood pressure was demonstrated at a dose of 1 to 10 mg of SG-1 per kg body weight (Figure 9). The mechanism of antihypertensive activity of SG-1 in the LEx is under investigation in our institute.

CONCLUDING REMARKS

In the past decade, there has increasingly been renewed and considerable interest in the ingestion of fermented dairy products containing viable *Bifidobacterium* and *Lactobacillus* to maintain a proper balance of normal intestinal flora and to enhance the beneficial relationship between host and intestinal flora. Although we are in a preliminary stage to understand the precise role of probiotics, scientific exploration of probiotic studies probably will help to develop feasible and practical measures to enhance the host's defence.

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