口服益菌療法促進急性腹瀉幼兒痊癒

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前言

腹瀉在先進國家仍是一主要問題，使得許多人必須去看醫生或住院。就病因學而言，輪狀病毒最常造成幼兒腸胃炎，特別在秋季和冬季。傳統上除了水份補充外，對此並沒有特別的治療方法。

目前，口服益菌療法為一新的治療方式，已引起小兒科醫師高度興趣。在這種療法中，乳酸菌和比菲德氏菌被廣泛地應用，這些口服乳酸菌和比菲德氏菌可短暫存在人体腸道內。口服益菌療法的特點包括增進營養利用，降低對乳糖過敏，減少有害病原菌，預防抗生素治療引起的腹瀉，避免輪狀病毒引起的症狀。

本試驗的目的在探討口服活的嗜酸乳酸桿菌及比菲德氏菌製劑對幼兒急性病毒性腸胃炎的臨床療效，同時評估上述製劑是否可以減少腹瀉次數及期間。

試驗方法

試驗期間：1999 年 10 月 ～2000 年 3 月
試驗人數：100 位住院乳幼兒。
年　　齡：6～60 個月
症　　狀：急性腹瀉持續 5 日以上，在 24 小時內，至少 3 次水便。
分組方式：第一組 (n=50)
投與 Infloran Berna,每次 1 膠囊，每 3 次，投與 4 日。
第二組 (n=50)
點滴補充液体，沒有投與任何藥物。

由護士記錄大便及嘔吐的次數及性狀。大便的性狀應記錄：水便、軟便或硬便。腹瀉期間以最後一次水便為準。

在第一組，第二日的 50 例糞便培養物中，祇有 20 例對乳酸菌或比菲德氏菌呈現陽性。這其中有 14 例呈現輪狀病毒陽性，6 例為陰性。因此在第二組以輪狀病毒陽性的前 20 例為控制組。

在單一個体中，有超過400種細菌株被檢測出來。因此，以一種適當的方法來確認目標菌株是很重要的。大便標本在1小時內以拭子收集，然後在PEA和BBE培養。在無氧箱中培養2天，再分離做純種培養。48小時後，選取G(+)菌，利用rapid ANA II system及code book來找出Lactobacilli及Bifidobacterium。

結果

100位乳幼兒經篩選加入本試驗。最後以20位為試驗組，20位為對照組。受試者年齡、在宅腹瀉期間，血中鈉濃度和脫水程度，兩組間沒有差異(表一)。

所有的乳幼兒都充分地給予營養和水分。沒有乳幼兒有慢性腹瀉。因為部分乳幼兒在入院未滿4天即已出院，所以祇比較第一日和第二日的腹瀉次數。第二日的腹瀉次數和第一日比較，有意義改善(表二)。同時，院內的腹瀉期間，試驗組也顯著減少達50%。

討論

* 1908年 Metchnikoff 提出假說：投與會產生酸的益生菌可以防止腹瀉和其他胃腸疾病。
* 經醱酵乳製品是常見的營養來源。在醱酵過程中降低乳糖濃度，增加了乳酸、半乳糖、胺基酸、脂肪酸和B群維生素。
* 嗜酸乳酸桿菌可促進乳糖醱酵，並且使結腸內的細菌在1~2天內，促進乳糖有效的利用。
* 嗜酸乳酸桿菌可增加大便的水分，改善便秘。
* 嗜酸乳酸桿菌和比菲德氏菌的利點：
  (1)預防院內感染急性腸胃炎。
  (2)預防抗生素引起的腹瀉。
  (3)治療復發的Clostridium difficili感染。
  (4)對嚴重燒傷患者，恢復腸內菌叢，並且防止腹瀉。
  (5)降低病毒的肆虐，並且延緩輪狀病毒感染的臨床發作。
* 在本試驗，我們先假設二者間沒有差異，然後使用Student's t test來比較二者間的差異。我們發現第二日和第一日腹瀉次數有明顯的改善($P<0.05$)，在院內腹瀉期間也顯著降低。
* 在本試驗，從大便中的細菌株分離出嗜酸乳酸桿菌的技術是困難的。在20個例中祇有2例嗜酸乳酸桿菌被分離出來(10%)。因此應有更好的方法來解決此一問題。

* 嗜酸乳酸桿菌在人体腸道內有許多作用，但它們祇在腸道中存活數天。所以希望找一個新的菌株能在腸道中存活較長時間，維持人体健康。

結論

口服乳酸菌和比菲德氏菌對輪狀病毒陽性或陰性的幼兒腹瀉皆有效，它降低腹瀉期間，並且能在腸道繁殖，促使腸道從腹瀉恢復正常，同時減少病毒肆虐，對急性腹瀉發作能夠安全地投與。

口服活菌療法促進兒童急性腹瀉之康復

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乳酸菌 (Lactobacilli) 在腸內可幫助人體對抗細菌及病毒的傳染。因此本文主探討嗜酸性乳酸菌 (*Lactobacillus acidophilus*) 及比菲德氏菌 (*Bifidobacterium infantis*) 對於住院病童之急性腹瀉是否有效果。本文共收集100名6個月到60個月大的急性腹瀉病童，隨意分為2組 (各50名)。實驗組病童每天服用三顆活菌膠囊，內含嗜酸性乳酸菌及比菲德氏菌各10^9隻活菌。對照組只接受點滴注射而不服用任何藥物。其中實驗組僅有20名病童可從糞便中培養出所用的兩種活菌。實驗組與對照組無論在年齡、住院前腹瀉時間長短、血鈉、血鉀及脫水程度均相類似。且實驗組與對照組在住院前一天的腹瀉頻率亦沒有統計上的差異。但在第一天及第二天住院腹瀉頻率上，實驗組有得到明顯統計上的改善。此外，在住院期間的腹瀉天數長短方面亦有縮短(3.1天與3.6天)。因此，口服活菌療法在兒童之急性腹瀉是一可能有效且安全的輔助療法。

關鍵字：嗜酸性乳酸菌，比菲德氏菌，輪狀病毒，急性腹瀉，口服活菌療法

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Oral Bacterial Therapy Promotes Recovery from Acute Diarrhea in Children

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*Lactobacilli in the intestines play an important role in developing natural defenses against both intestinal bacterial and viral infections. So a prospective clinical study was carried out at Cathay General Hospital to determine the effect of Lactobacillus acidophilus and Bifidobacterium infantis on the course of acute diarrhea in hospitalized children. Altogether 100 children between 6 and 60 months of age were collected and randomly allocated into 2 groups. Study group (n=50) was given Infloran Berna, which contains 10^9 viable Lactobacillus acidophilus and 10^9 Bifidobacterium infantis, one capsule tid for 4 days and control group (n=50) received parenteral rehydration only without any medication. Only 20 stool cultures in study group had positive culture results for Lactobacillus or Bifidobacterium. All children were evaluated for the degree of dehydration before rehydration. The clinical course of diarrhea was followed during the treatment period. Features on admission were similar between the study group and control group in age, duration of diarrhea at home, serum sodium & potassium and dehydration degree. The duration of diarrhea was defined as the time until the last appearance of watery stool. There was no difference between the study group and control group in the frequency of diarrhea stools on the day before admission ($p > 0.05$). However, the frequency of diarrhea for study group improved on the first and second day of hospitalization with statistical difference ($p < 0.01$). The duration of diarrhea during hospitalization in study group also decreased (3.1 vs. 3.6 days, $p < 0.01$). Oral bacterial therapy is an effective adjuvant therapy in rotavirus positive and negative children with diarrhea and can safely be administered during an episode of acute diarrhea. (Acta Paediatr Tw 2001; **42**:301-5)*

Key words: lactobacillus acidophilus, bifidobacterium infantis, rotavirus, acute diarrhea, oral bacterial therapy

INTRODUCTION

Diarrhea is still a major problem in industrialized countries resulting in many clinic visits and hospital admissions. In developing countries, children experience an average of 3.3 episodes of diarrhea per year.[1] In Taiwan, diarrhea occurs in about 11.4% of pediatric admissions.[2] About one-fourth of avoidable deaths in hospitalized children is a result of diarrhea and 80% of these deaths occur in children less than 2 years old.[1] Rotavirus is the most common cause of children's gastroenteritis especially in autumn and winter .[3] There is no special treatment for rotavirus infection except for rehydration[4] and supportive treatment including soy-based , lactose-free formula.[5]

An alternative therapeutic method using oral bacterial therapy is now generating great interest among pediatricians.[6] The Lactobacillus strain and Bifidobacterium strain are the most widely used agents in this method.[7,8] *Lactobacillus* and *Bifidobacterium* remain transiently in the human intestine after oral administration.[9,10] The advantage of oral bacterial therapy includes increased nutrient utilization, alleviation of lactose irritability, decrease of pathogenic bacteria,[10] treatment of hepatic encephalopathy,[9] prevention of antibiotic associated diarrhea[11] and

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shedding of rotavirus.[12]

The purpose of this study was to investigate the clinical efficacy of oral administration of live *Lactobacillus acidophilus* and *Bifidobacterium infantis* in children with acute viral gastroenteritis and to find out whether *Lactobacillus* and *Bifidobacterium* could reduce the duration and frequency of diarrhea.

PATIENTS AND METHODS

The study was carried out between October 1999 and March 2000 to cover a rotavirus epidemic season. A total of 100 children, from 6 to 60 months old were consecutively admitted to our department for acute diarrhea with a duration of less than 5 days and with more than 3 watery stools during the previous 24 hours. The following subjects were excluded: (1) children with bloody stool (2) children taking antidiarrheal drugs or antiperistaltic drugs either before or after admission to the hospital (3) children receiving lactose-free, protein hydrolysated formula for a malabsorptive disorder (4) immunocompromised children. We used Taita No. 2 injection solution (which contains sodium 40mEq/L, potassium 12 mEq/L, chloride 26 mEq/L, acetate 20 mEq/L, phosphate 6 mM /L, glucose 33 g/L; Otsuka pharmaceuticals, Taiwan) to replace the dehydration and maintained proper fluid supply. After initial fluid therapy , the children were randomly allocated to receive one of the two regimens . Study group (n=50) was given parenteral fluid supply (Taita No. 2 solution) and Infloran Berna(Rehhagstrasse 79, CH-3018 Berna Switzerland) ,which contains two types of live bacteria : *Lactobacillus acidophilus* (Fig.1) and *Bifidobacterium infantis* (Fig.2) 1 capsule tid for 4 days. There are 10^9 viable *Lactobacillus acidophilus* and 10^9 *Bifidobacterium infantis* in each capsule of Infloran Berna. Control group (n=50) was given the same parenteral rehydration only without receiving any medication. Because these patients of both groups all had vomiting symptom and could not take oral rehydration well, parenteral rehydration was given to all these patients. After the vomiting symptom improved, the patients began to try oral feeding. There were no difference between these two groups on food. They took rice, toast or half strength infant formula. There were no restrictions on the amount of food. The patients were evaluated for degree of dehydration and daily weight.

Fig.1. *Lactobacillus acidophilus* cultured from Infloran Berna. (Gram stain, 10×100)

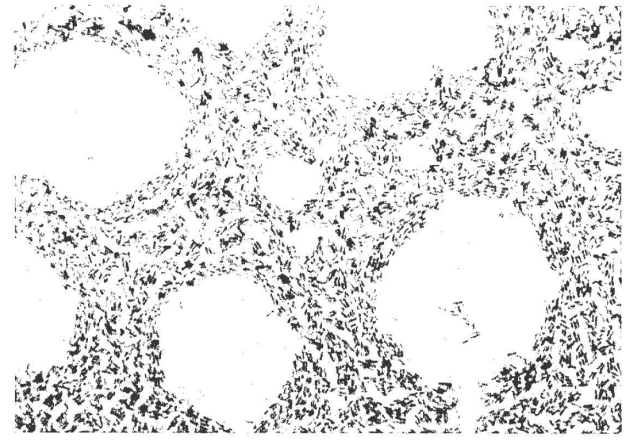

Fig. 2. *Bifidobacterium infantis* cultured from Infloran Berna. (Gram stain, 10×100)

The number and the quality of the stools and vomitus were followed by attending nurses. The stool was recorded as watery, loose or solid. The duration of diarrhea was defined as the time until the last appearance of watery stools. The recovery was defined as no more watery stool appearance. All children were well nourished and hydrated before they were discharged. There were no children with chronic diarrhea.

Among the 50 stool cultures on the second day in study group, only 20 cases had a positive culture result for *Lactobacillus* (Fig. 3) or *Bifidobacterium* (Fig. 4). There were 14 rotavirus positive and 6 negative in these 20 cases. The rotavirus infection was detected by using the latex agglutination test.

A stool specimen was collected by anaerobic swab within one hour of defecation. Then it was cultured on PEA (phenyl-ethyl-alcohol) and BBE (bacteroides bile esculin) media. After culture for 2 days , one re-isolation was done for pure culture. After 48 hours, we selected

the Gram(+) bacilli and used the rapid ANA II system which uses a biochemical method to identify anaerobic bacteria from Remel Inc. (12076 Santa Fe Drive, Lenesa, Kansas 66215-3594 USA) and the code book to identify the *Lactobacillus* and *Bifidobacterium*.

In this study, we used Student's t-distribution and χ^2 test for statistical analysis.

RESULTS

A total of 100 children were collected in this study. The age, duration of diarrhea at home, serum sodium & potassium and dehydration degree during hospitalization were similar in the study and control groups (Table 1).

Some children were discharged before the fourth hospital day, so we only compared the frequency on the first and second hospital days. There was no difference between the study group and control group in the frequency of diarrhea on the day before admission. The frequency of diarrhea improved markedly (Table 2) on the first and second day of hospitalization and this was statistically significant($p<0.01$). In addition, the duration of diarrhea during hospitalization also decreased (3.1 v.s 3.6 days) in the study group which was also statistically significant ($p< 0.01$).

The recovery rate on the second day was 52% in study group and 18% in control group which was statistically significant ($p< 0.01$).

DISCUSSION

The hypothesis that ingestion of acid-producing probiotic bacteria could be used to prevent diarrhea and other intestinal disease was first suggested by Metchnikoff in 1908.[12] The first study using the *Lactobacillus* strain in the treatment of acute diarrhea was reported by Isolauri et al[7] in Finland. In that study,

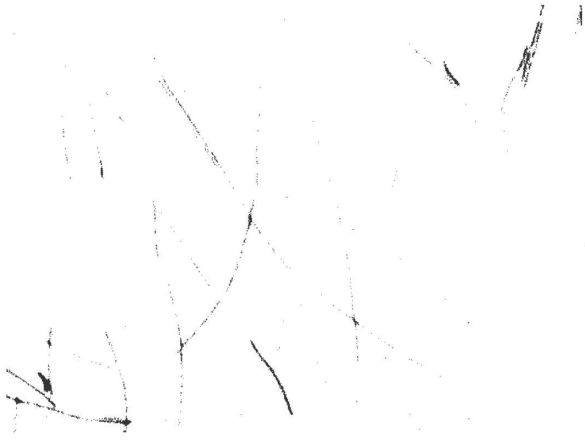

Fig. 3. *Lactobacillus* isolated from stool of patient in study stain, 10×100)

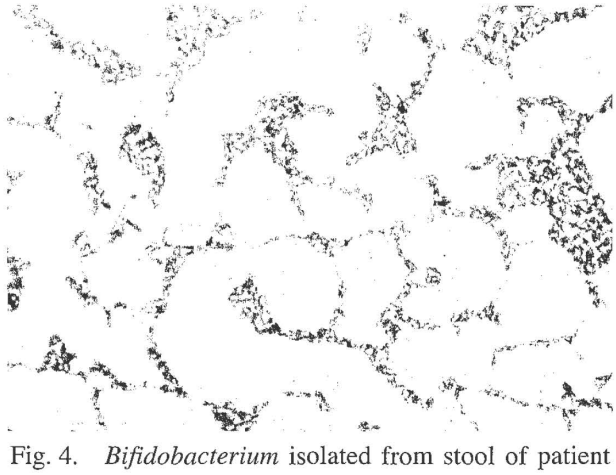

Fig. 4. *Bifidobacterium* isolated from stool of patient in study group.(Gram stain, 10×100)

Table 1. Clinical Characteristics of Study and Control Groups

	Study group(50)		**Control group(50)**		
	mean	SD	mean	SD	*p*
Age(m/o)	17.2 (6.1-58.5)	11.5	14.5 (6.5-57.2)	10	0.16
Duration of diarrhea at home(day)	1.6 (0-4)	0.9	1.66 (0-4)	0.6	0.51
Serum Na(mEq/L)	137.4(129-146)	3.5	136.8 (126-145)	3.7	0.14
K(mEq/L)	4.1 (3.0 -5.3)	0.5	4.0 (3.1-5.0)	0.6	0.38
Dehydration(%)	4.3 (3-6)	1.5	4.0 (3-6)	1.4	0.32
Male/Female	30/20		28/22		

Table 2. Outcome of Therapy

	Study group(50)		Control group(50)		*p*
	mean	SD	mean	SD	
Diarrhea frequency					
Day 0	6.7 (3-16)	2.6	6.8 (3-18)	3.1	0.64
Day 1	2.9 (0-10)	2.0	4.0 (0-9)	1.3	<0.01
Day 2	1.9 (0-9)	1.9	3.7 (0-11)	2.4	<0.01
Duration of diarrhea in hospital (day)	3.1 (1-3)	0.7	3.6 (1-6)	0.8	<0.01
Recovery rate on the 2nd day	52%		18%		<0.01

the majority of children had rotavirus gastroenteritis . Fermented dairy products are a source of nutrients. The fermentation process reduces the concentration of lactose and increases the concentration of lactic acid , galactose, free amino acids, fatty acids and certain B vitamins.[13] *L.acidophilus* may enhance lactose fermentation and make colonic bacteria adapt quickly to lactose within 1 to 2 days, causing efficient utilization of lactose.[14] *L.acidophilus* can also help to increase the fecal moisture which can help to improve constipation. [10]

Rotavirus gastroenteritis gives rise to biphasic diarrhea[15] , the first phase being an osmotic diarrhea and the second phase being related to overgrowth of bacteria. The osmotic diarrhea is induced by reduced jejunal disacharidase activity, particularly lactase, direct mucosal damage and inhibition of mucosal carbohydrate transport.[16]

The mechanism by which oral bacterial therapy acts on recovery from gastroenteritis is unclear. Many studies have shown that fermentation with *Lactobacilli* increases the quantity, availability and digestibility of nutrients[17], and inhibits the adhesion of enterotoxigenic *Escherichia coli* fimbriae to piglet ileal mucus.[18] In addition, *Lactobacillus acidophilus* produces a substance with potent inhibitory activity against a wide range of bacterial species. It inhibited anaerobic bacteria including *Clostridium* spp., *Bacteroides* spp., members of the family *Enterobacteriaceae*, *Pseudomonas* spp., *Staphylococcus* spp. and *Streptococcus* spp. in one report.[19] Thus, oral bacterial therapy helps to promote the balance of osmotic insult and appears to be a promising means to counteract the disturbed microbial balance.

Some studies[3,20] in mice have shown that *Lactobacilli* enhanced phagocytic and lymphocytic activity. Studies of oral therapy with a viable *Lactobacillus* strain in Finland[21-23] indicated that *Lactobacillus* increases the IgA specific antibody-secreting cell response to rotavirus. Thus it promotes recovery from rotavirus diarrhea and augmentation of the local immune defense. Moreover, the specific IgA also protects the patient against reinfection.[24]

The above mechanisms can all account for the reason why oral bacterial therapy helps promote recovery from rotavirus gastroenteritis. However, the efficacy of oral bacterial therapy in children without rotavirus could be related to an antimicrobial substance that inhibits the growth of Gram-negative and Gram-positive bacteria.[19] Oral bacterial therapy was effective both in patients with rotavirus gastroenteritis and in those whose fecal samples were negative for rotavirus.[6]

We found that the diarrhea frequency on the first day and second day improved ($p<0.01$) and the duration of diarrhea was reduced with statistical difference ($p<0.01$).

There was one unpublished data of clinical investigation of Infloran Berna in Taiwan by Chou in 1989. It showed good therapy results. Another unpublished data of Infloran Berna in Taiwan was focused on nontyphoid Salmonellosis by Chao. It also showed good results on hospitalized patients.

This study focused on hospitalized patients. Ambulatory patients who were less severely ill and were under observation as outpatients also benefitted from this therapy in Alfredo's study in 1979.[6]

We had difficulty in isolating *L. acidophilus* from the bacterial species in the stool in this study. So only 2 *L. acidophilus* were isolated in the study group (4%). A better method is needed to conquer this problem.

L. acidophilus has many advantages in the human

digestive system but it only survives in the gastrointestinal tract for a few days.[9] A new strain which could colonize in the human gut for a longer time would be advantageous.

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