行政院國家科學委員會專題研究計畫　成果報告

極低體重早產兒口服原生素對壞死性腸炎的影響

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行政院國家科學委員會補助專題研究計畫■ 成 果 報 告
□期中進度報告

「極低體重早產兒口服原生素對壞死性腸炎的影響」

Impact of oral Probiotics on Necrotizing Enterocolitis for Preterm Extremely Low Birth Weight Infants

計畫類別：■ 個別型計畫　　□ 整合型計畫
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執行單位：中國醫藥大學附設醫院 兒科部

中　華　民　國　95　年　10　月　1　日

中文摘要:

研究宗旨：我們研究在極低體重早產兒(VLBW) 以益生菌減少壞死性腸炎(NEC)發生率的有效性。

患者和方法：我們假設益生菌 Infloran（含 Lactobacillus acidophilus 和 Bifidobacterium infantis）可以減少極低體重早產兒（出生重量＜1500 gm)的 NEC 或死亡率。從 2005 年 4 月 1 日，在八個新生兒加護病房進行一個前瞻、遮蓋、隨機化的研究。存活且有腸道餵食的 VLBW 才納入試驗。實驗組給母乳或混合哺乳與 Infloran125/毫克/公斤/劑，每日兩次-共六週；控制組只餵食母奶或混合哺乳。母奶來自嬰兒母親或母奶銀行，所有母奶都由不參與嬰兒照護的小組準備，且遵從來自密封的醫囑；臨床照護者嬰兒不知嬰兒組別。主要結果是測量死亡或 NEC（≧stage 2)的發生率 。

結果：共有三百二十個嬰兒納入研究，163 人在實驗組和 157 人在控制組。人口特徵和臨床變項在兩組是相似。死亡或 NEC 的發生(≧階段 2）在實驗組顯著較低(3/163 對 10/157)。與控制群組比較實驗組 NEC 的發生(≧ 階段 2）也是顯著的低（3/163 對 7/157)。沒有血液培養長出乳酸桿菌屬或比菲德氏菌也沒有發現其它副作用。

結論：以 Infloran 為益生菌哺餵極低體重早產兒 6 個星期可以減少 NEC 的發生。

關鍵字：*益生菌、壞死性腸炎、極低體重早產兒*

ABSTRACT

Objective: We investigated the efficacy of probiotics in reducing the incidence of necrotizing enterocolitis (NEC) for very low birth weight (VLBW) infants.

Patients and Methods: A prospective, masked, multi-center randomized control trial was conducted at eight neonatal centers to evaluate the beneficial effects of probiotics for NEC among VLBW (<1500 g) infants. VLBW infants who started to feed enterally were eligible and were randomized into 2 groups after parental informed consents were obtained. Infants in the study group were fed with Infloran (Lactobacillus acidophilus and Bifidobacterium bifidus) with breast milk or mixed feeding (breast and formula) twice daily for 6 weeks. Infants in the control group were fed with breast milk or mixed feeding. The clinicians caring for the infants were blinded to the group assignment. The primary outcome measurement was death or NEC (□stage 2).

Results: Three hundred and twenty infants were enrolled: 161 in the study group and 157 in the control group. The demographic and clinical variables were similar in both groups. The incidence of death or NEC (□ stage 2) was significantly lowerin the study group (3 of 163 vs 10 of 157). The incidence of NEC (□ stage 2) was also significantly lower in the study when compared with the control group (3 of 163 vs 7 of 157). None of the positive blood culture grew Lactobacillus or Bifidobacterium species. No other adverse effect was noted.

Conclusion. Infloran as probiotics fed enterally to preterm VLBW infants for 6 weeks reduces the incidence of NEC.

Keywords: probiotics, necrotizing enterocolitis, very low birth weight infants.

ABBREVIATIONS. NEC, necrotizing enterocolitis; VLBW, very low birth weight.

報告內容：

Introduction

Necrotising enterocolitis NEC is one of the most common gastrointestinal emergencies in preterm very low birth weight (VLBW) infants with highly variable incidence affecting 7% to 14% of these infants; [1] Recent reports suggest increasing occurrence of NEC, estimating up to 9000 cases of NEC in the United States every year, with death occurring in 15 % to 30% of affected individuals. [2]

As the survival rate of VLBW infants increases, NEC remains a critical eminent problem.

The pathogenesis of NEC is unknown, but is widely considered as a multi-factorial disease; prematurity, enteral feeding, intestinal hypoxia-ischemia, and bacterial colonization are considered major risk factors. [3] Most likely, NEC is the clinical culmination of multiple different risk factors interacting with each other to produce bowel injury through a final, common inflammatory pathway. Researches showed that platelet aggregation factor (PAF) interleukin-1.6.12.18 (IL-1, IL-6, IL-12, IL-18) and tumor necrosis factor- (TNF -) [4-8] and anti-inflammatory cytokines such as interleukin 4.10 (IL-4.10) [9.10] by intestinal epithelial cells is highly relevant to be involved in the final common pathway of NEC. [4-11]

Recent studies indicated that commensal bacteria block ubiquination and degradation of inhibitory factor that results in very low level of inflammatory response continuously at mucosa.[12.13] Unfortunately pathogenic floras attach to the epithelium cell of preterm infants much easier than to the full term infants and the fetal enterocyte have exaggerated IL-8 response.[14] It has bee thought that inappropriate, accentuated inflammatory response to colonizing pathogenic flora at premature gut plays a major role, the inflammatory cascade further promotes spread of bacteria or toxin, and end up with ischemia, necrosis and even perforation. [15-17]

We and Bin-Nun had proved that probitics reduces the incidence and severity of necrotizing enterocolitis in preterm VLBW infants. [18.19] Nevertheless, there is no solid data in the

literature as to the optima strains, timing, dosage and duration of probiotics administering to preterm VLBW infants at preset; and these need further investigate. Bifidobacteria and lactobacilli are commonly found in breast-fed infants; [20] and most of the NEC in VLBW infants occurred before 6-8 weeks of age; [21]; we therefore hypothesized that oral probiotics contains Bifidobacteria and lactobacilli fed to preterm VLBW infants for 6 weeks would reduce the incidence and severity of NEC.

PATIENTS AND METHODS

From April 1, 2005 to Oct 31, 2006, a prospective masked randomized control trial was conducted at the level III Neonatal Intensive Care Unit (NICU) of eight medical centers in Taiwan. Study protocol was approved by Institutional Review Board (IRB) of each hospital. Preterm VLBW infants (birth weight < 1500 gm) who were fed enterally were eligible for the trial. They were randomized into the study or control group by a random number table sequence after informed parental consents were obtained. The allocations were in sequentially numbered and were sent by computer center located at the children hospital of China Medical University when the participate hospital registered. Preterm VLBW infants who had severe asphyxia (stage III), fetal chromosome anomalies, fetal cyanotic congenital heart disease, congenital intestine atresia, gastroschisis, omphalocele, exclusive formula feeding and nothing per oral was more than 3 weeks were excluded.

The clinicians who provided care to the infants were blinded to the grouping. Investigators were not involved in the care of these infants. Study group was fed with infloran (Lactobacillus acidophilus and Bifidobacterium bifidus, Swiss Serum and Vaccine Institute Berne, Switzerland) 125mg/kg/dose (CFU 10^9) twice daily with breast milk or mixed feeding (breast and formula) for 6 weeks; the control group was fed with breast milk or mixed feeding. Infloran was stored in refrigerator at a tempreture between 2 – 8^0 C and mixed with breast milk or formula before feeding. Breast milk was either from infant's own mother's milk or from breast milk bank. Both breast milk of the study and control group were prepared by breast milk team who were not involved in the care of the infant and follow the order from a sealed envelop. Feeding was started

when the infant's vital signs were stable, had active bowel movement, without abdominal distension, without bile or blood from the oral-gastric tube, and did not have an umbilical artery or umbilical venous catheter in place for at least 24 hours.

A strict feeding protocol was followed for all study infants. Depending on the birth weight and gestational age, a certain amount of breast milk was initiated after the infant tolerated one trial of distilled water. On the first day, 1 mL/kg - distilled water was given twice, followed by breast milk. The amount of feeding was advanced slowly if tolerated, with no more than 20 mL/kg/day of increment per feed. An oral intake of 100 mL/kg/d was defined as complete enteral feeding. Feeding was stopped if there was any sign of feeding intolerance, defined as the presence of gastric aspirate in the amount that was more than half of the previous feeding, twice, with abdominal distension. Infants who weighed under 1,000gm received total parenteral nutrition (TPN) until half of the calories were supplied by oral route. The same attending physician was in charge of the care of the infants during their hospital stay. The residents who rotated through the NICU provided the care following established protocols in the unit. Definition of prenatal steroid, small for gestational age, prolonged rupture of amniotic membrane, chorioamnionitis, asphyxia, respiratory distress syndrome, patent ductus arteriosus, intraventricular hemorrhage (IVH), sepsis; indication of surfactant and indomethacin were described as in our previous study. [18] There were no modifications in any management protocols, clinical practices, equipment, infrastructure, or any other elements in each unit during the study period. All the definitions and protocols got consensus among the eight centers after several discussions before the study was begun.

NEC was classified by modified Bell's classification. [22]The final determination of the diagnosis of NEC (≧ stage2) was made by two independent attending physicians who did not know the group assignment of the infant. Demographic and clinical variables that were potential risk factors for NEC were prospectively abstracted from the medical records. These factors were as our previous study except added antibiotics use per weeks and weight gain per week; these factors were prospectively abstracted from the medical records.18

Primary outcome measurement was the incidence and severity of NEC (≧ stage 2); or death. Secondary outcome were culture proved sepsis, chronic lung disease, periventricular leukomalacia (PVL), weight gain per week, duration of TPN, lengthy of stay.

SAMPLE SIZE CALCULATION AND STATISTICS

The recent data showed that the combined incidence of NEC (≧stage 2) or death was about 26 % in Taiwan. [23] Setting the α error <0.05 and β error <0.2, and an absolute reduction of the incidence of NEC or death by 50%, the number needed to verify our hypothesis was 264 by two-tail.

Chi-square test was used to examine the association between categorical risk factors and mortality when its assumption was held (less than 20% of cells had expected values of less than five), whilst a two-tailed Fisher*s exact test was used when assumption of Chi-squared test was not held. Student*s t-test was used to explore the differences in continuous risk factors.

RESULTS

There were 413 VLBW infants admitted to the eight NICU during the 1.5-year study period. Of these infants, 95 were either expired (n =69) or had met the exclusi on creteria before enrolled to the study (n = 9) or the family members declined consent for study (n = 17). A total of 320 infants were enrolled in the trial: 163 in the study arm and 157 in the control arm. Fifty-six infants in the study group and 61 infants in the control group were fed with mixed feeding. The maternal clinical and infant's demographic and clinical characteristics did not differ between the 2 groups (Table 1). The infants' clinical characteristics also did not differ between the 2 groups (Table 2). Table 3 shows the outcomes of the study by logistic regression analysis. The incidence of death or NEC was significantly lower in the probiotics group when compared with the control group (3 of 163 [1.8 %] vs 10 of 157 [6.4 %], respectively; P .009). The incidence of NEC was also lower in the probiotics when compared with the control group (3 of 163 [1.8%] vs 7 of 157 [4.5 %], respectively; P .04).

There were 4 cases of severe NEC (stage 3) in the control group and one in the probiotics group (P .03 by bivariate analysis). The incidence of culture proved sepsis, CLD, PVL were no

significant difference between both groups. None of the positive blood cultures grew Lactobacillus or Bifidobacterium species. No other adverse effect was noted. The administration of infloran to the study group resulted in a significantly earlier colonization of Lactobacillus acidophilus and bifidobacteria (8.1 +/- 3.9 days of life) than in the control group (11.3 +/- 4.7 days of life). On day 7 after infloran, Lactobacillus acidophilus and bifidobacterial dominance (> 90% of the intestinal microflora) could be found in 26% of infants in the study group and only in 2% of the control group ($p < 0.001$).

DISCUSSION

This multi-center control trial showed that probiotics as Infloran reduces the incidence of NEC or death for preterm VLBW infants. We also found that the study group had a lower incidence of NEC and sepsis. We found that bifidobacteria and lactobacilli were more prominent with less pathogenic microorganisms in stool culture of the study group compared to the control group. According to our data, the number needed to treat to prevent 1 case of NEC is 32, and the number needed to treat to prevent 1 death due to NEC is 29.

Although many variables are associated with development of NEC, only prematurity and low birth weight have been consistently identified in case controlled studies.[24.25]Many other factors that were associated with an increased risk of NEC had been reported; [24.25] because the current multi-center study was designed as a randomized, controlled trial, all the risk factors were distributed randomly and showed no difference between the 2 study groups.

A major component of the proposed pathogenesis of NEC is the interaction of bacteria with the premature gut. [26-33] Sakata H and Gewolb IH found that the Bifidobacteria were undetectable in the intestinal flora during the first 1 to 2 weeks after birth and did not predominate until after the third week of life among VLBW infants even receiving breast milk. [26.27] Lawrence and Shah demonstratedthat delay gut colonization in NICU, make the intestine absorbed of intact bacterial toxin, which damaged the immature ileum and developed NEC.[28.29] Hoy andMillar found that there were quantitative and qualitative changes in the fecal flora before NEC.[30.31] Panigrafi and Dai showed that bacterial translocation leading to NEC.[32.33] All these studies

suggest a strong relationship of delay and low colonization of commensal flora and proliferation of pathogenic flora in the immature gut that predisposing preterm infants to develop NEC. Using animal model Caplan and Butel showed that Bifidobacteria supplement in neonate rat and quail model resulted inintestinal colonization and subsequently reduction in NEC-like lesion.[34.35] Bifidobacteria and lactobacilli could break down ingested sugars to form lactic acid, inhibite pathogenic microorganisms, not undergo translocation, producing protective nutrients (arginine, glutamine, short-chain fatty acids) protective against the translocation of other bacteria. [36] These characteristics make that probiotics contained Lactobacillus and Bifidobacterium become best shot for preventing of NEC.

There were five clinical trials till now that evaluated the impact of probiotics on NEC. In Hoyos,s [37] historical control study, infloran was given to all infants admitted to NICU and showed significant reduction in the incidence of NEC and NEC associated death. Dani performed a multi-center study double blind control trial involving 585 preterm VLBW infants with LGG as probiotics in Italy. [38]There were no significant differences between the probiotic and placebo groups in regards to any of the three outcome variables, including NEC. However, the event rate was low for all three variables and needed a much larger sample size to verify their hypothesis. A recent study by Dr. Bin Nun from Israel and published in Journal of Peds showed similar results as our previous study with significant reduction of death or NEC using Bifidobacteria infantis, Streptococcus thermophillus, and Bifidobacteria bifidus. 19

Although the four control studies were different somewhat in study design and methodology, the four randomized trials can be compared because the Breslow–Day test shows that the relative risk assessments were homogeneity. With method of Mantel– Haenszel, the weighted, pooled estimate of relative risk is 0.34, with a 95% confidence interval of 0.20 to 0.90, suggesting a beneficial effect of probiotic treatment on reducing the incidence of NEC. The weighted risk difference summarising the presented studies, 0.021, indicates that the number needed to treat to prevent one case of NEC is 41 infants.

The probitics we used in the current study was different from our previous study

(Bifidobacteria infantis was changed to Bifidobacteria bifidus); although the results of ours two studies seemed similar, the previous study did not have stage III NEC. It seemed that Bifidobacterium infantis might be more effective to prevent NEC. Evidence disclosed that oral administration of specific strains of Lactobacillus species, stimulated the Bifidobacterium microbiota; [39] Combination of this type of probiotic microbes with breastfeeding have synergistic effect on gut humoral immunity and microbiota modification. Giving that Bifidobacterium infantis, B. longum and B. breve are the most common strain in health breast feeding infants, [40] probiotics which contains these three Bifidobacterium might be more appropriate to prevent NEC.

Breast milk and even donors breast milk could reduce the incidence of NEC, but breast milk along could not eradicate NEC.[41] Evidence showed that those infants who fed breast milk still got NEC was because IL-10 deficiency; [42]probiotics could induce IL10 production, this may explained partially why that breast milk has synergistic effect with the Bifidobacteria and Lactobacilla to inhibit the inflammation response of NEC.

Because there were very few preterm VLBW infants fed with formula milk alone; in reality, it will be extremely difficult to conduct the study adopting formula milk with probiotics.

Although some reports demonstrated that probiotics reduced the incidence of sepsis.[43]This current study didn't showed that probiotics reduced the incidence of sepsis in VLBW infants as in our previous study.18 This might result from the the different enrolled time and the short period of probiotics usage.

Many clinicians worry the safety of probiotics treatment in immunodeficient hosts such as neonates. Others and we did not observe complications (such as Lactobacillus or Bifidobacterium sepsis) due to probiotics during study. [18.19.40] The number accumulated from all the clinical trial might be power to state that it is relative safe if we compare the incidence and disaster of NEC (7-14%) and the possible sepsis due to probiotics (0/715, sum of the clinical trial) for VLBW infants.

Most stably healthy colonic and vaginal flora include 2 species of lactobacilli; [44] and

experience from clinical studies show that the efficacy varies from none to significant as the treatment expands from single-strain to full flora replacement single-strain probiotic is less effective than multistrain probiotic.[45] we therefore suggest that multiple strains contains Bifidobacterium and Lactobacillus are superior to single strain; probiotics should be used as early as possible and a dosage of around 10^9 is enough to colonize in the intestine. Because most of the NEC in VLBW infants occurred before 6-8 weeks of age, therefore, we suggest that a 6-8 weeks period of probiotics should be adequate to prevent NEC.

The issue of prevalence of atopic disease such as atopic dermamitis[46], or allergic rhinitis [47] in those preterm infants fed with probiotics would be very interesting; if probiotics indeed reduces the incidence of atopic diseases in infancy, these observation would further extend the rationale of treating preterm VLBW infants with probiotics.

We observed 6 infants developed NEC without enteral feeding and before enrolled to the study, 5 of them were weighted less than 1000 g. Probiotics alone could not eliminate NEC. Future study should focus on these victims; immediate oral probiotics soon after birth or probiotic mixed with amniotic fluid that contains many growth factors [48]might be the ideal silver bullet to prevent NEC effectively.

We concluded that oral Infloran as probiotics administration for 6 weeks reduces the incidence of NEC in preterm VLBW infants.

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計畫成果自評：

本研究評估極低體重早產兒服用 INFLORAN 後 NEC 或死亡的發生率，共有三百二十個嬰兒納入研究，163 人在實驗組、157 人在控制組，人口特徵和臨床變項在兩組是相似。死亡或 NEC 的發生(≧階段 2) 在實驗組顯著較低 (3/163 對 10/ 157)。與控制群組比較實驗組 NEC 的發生(≧ 階段 2) 也是顯著的低 (3/163 對 7/154)。沒有血液培養長出乳酸桿菌屬或比菲德氏菌也沒有發現其它副作用。

結論：以 Infloran 為益生菌哺餵極低體重早產兒 6 個星期可以減少 NEC 的發生。至於其他次要的指標如慢性肺病的發生率，細菌培養證實的敗血病、每星期增加的體重、住院天數則沒有差別，可惜因為研究經費不足必須結案，但統計上仍有個案數不足的疑慮。

附件一、

Table1. Maternal and Clinical and Infants demographic and clinical characteristics

characterisitcs	Control group (N= 157)	Study group (N= 163)	P-value
Prolonged rupture of amniotic membrane, n (%)	45	44	1.00
Preeclampsia, n (%)	21	25	0.73
Prenatal steroid, n (%)	70	57	0.41
Mutipregnancy, n (%)	46	32	0.14
Chorioamnionitis, n (%)	8	7	0.92
Male, n (%)	91	86	0.39
Small for gestational age, n (%)	126	120	0.17
Gestation, wk	28.55±2.58	28.09±2.78	0.13
Birth weigh, g	1117.30±246.47	1029.40±258.00	0.00
Apgar (5 min)			
<3	30	29	
4-6	24	50	0.00
>7	102	83	
PH	7.29±0.10	7.26±0.13	0.03
Age at enrollment,*d	6.19±5.70	5.94±3.19	0.65
Antibiotics use during 1st wk	135	145	1.00
Antibiotics use during 2nd wk	60	71	0.50
Antibiotics use during 3rd wk	43	54	0.44
Antibiotics use during 4th wk	34	43	0.55
Antibiotics use during 5th wk	30	41	0.40
Antibiotics use during 6th wk	23	30	0.70
Ues of surfactant, n	74	97	0.03
Umbilical artery catheter, *d	0.63±1.44	0.97±1.75	0.06
Umbilical venous cathter, *d	1.14±2.06	1.56±2.27	0.09
Intermittent mandatory ventilation, *d	13.77±23.61	14.64±21.49	0.74
Pneumothorax, n (%)	0	4	0.14
Use of dopamine, n (%)	66	79	0.51
Dopamine, d	2.48±5.83	2.44±3.85	0.95
Indomethacin, n (%)	63	90	0.01
IVH >3	6	5	0.96
Age onset of NEC*	3	1	0.29

Table2.impact of Probiotics on feeding amount and weight gain

Variables	Control group (N= 157)	Study group (N= 163)	P-value
Total parenteral nutrition, d	10.63±16.44	13.95±15.57	0.07
Feeding amount at 42 d	139.09±67.57	148.20±74.90	0.32
Full feeding day	28.80±18.34	26.30±18.44	0.24
Body wt gain at 14d	64.94±65.36	49.97±56.36	0.03
Body wt gain at 28d	115.54±73.81	107.02±69.20	0.30
Body wt gain at 42d	149.56±86.70	127.50±83.49	0.03

Table.3 Outcome variable after oral probiotics

Variables	Control group (N= 157)	Study group (N= 163)	P values
Death or NEC	10	3	0.04
Death	3	0	0.05
NEC grade 2 or 3	7/154	3/163	0.04
Sepsis (culture proven)	21	34	0.09
NEC or sepsis	5	3	0.68
CLD	30	40	0.27
PVL	5	8	0.62