

A randomised controlled trial of the probiotic *Bifidobacterium breve* BBG-001 in preterm babies to prevent sepsis, necrotising enterocolitis and death: the Probiotics in Preterm infantS (PiPS) trial

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Scientific summary

The Probiotics in Preterm infants (PiPS) trial

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Scientific summary

Background

Necrotising enterocolitis (NEC) and late-onset sepsis remain important causes of mortality and morbidity in the preterm infant. The postnatal acquisition of diverse bowel flora is delayed in the preterm infant; this may contribute to reduced barrier properties of the intestinal mucosa, making invasion and/or translocation of the bowel wall by potentially pathogenic bacteria more likely. The hypothesis underpinning studies of probiotic administration in preterm babies is that, by encouraging the bowel flora to resemble that of a healthy breast-fed full-term infant more closely, barrier function will be improved and the incidence of late-onset sepsis and NEC will be reduced. The most recent Cochrane review of this topic includes 20 randomised trials with > 5500 participants. The meta-analysis suggests that probiotics do not reduce sepsis but are associated with statistically significant reductions in NEC incidence and all-cause mortality; no adverse events were reported in any trial and the recommendation was made that probiotics should be given routinely to preterm infants, including those whose birthweight is < 1 kg. Despite this, the use of probiotics is variable. Concern has been expressed about the rigour of a number of the published trials, the heterogeneity of the participants, particularly in respect of exclusions and rates of NEC and death in the placebo groups, and the wide range of interventions. These live microbial products are likely to cross-colonise babies, yet none of the trials systematically reports stool colonisation by the administered strains in either the active intervention or placebo groups. This leaves clinicians uncertain about the benefit of routine use and with little guidance as to choice of product.

Objective

To evaluate efficacy and safety of *Bifidobacterium breve* strain BBG-001 to reduce NEC incidences, late-onset sepsis and death in an unselected population of preterm infants in England.

Design

Double-blind, placebo-controlled trial.

Setting

Hospitals with tertiary or secondary neonatal intensive care units in and around London. Recruitment took place in 24 hospitals and continuing care prior to the initial discharge from hospital in a further 33 hospitals.

Participants

Babies born between 23⁺⁰ and 30⁺⁶ weeks' gestation were randomised within 48 hours of birth. The randomisation program used a minimisation algorithm to ensure balance on hospital, sex, gestational age and whether or not randomisation occurred within 24 hours of birth. Multiple births were randomised individually. Those with potentially lethal malformations or any gastrointestinal malformation apparent within 48 hours of birth or no realistic chance of survival were excluded.

Interventions

The active intervention, *B. breve* BBG-001, was provided in single-dose sachets as a powder, freeze-dried with maize starch. The placebo was maize starch alone provided as an identical powder in identical sachets. The interventions were suspended in 3 ml of one-eighth strength of the 'elemental' formula Neocate® (Nutricia Ltd, Trowbridge, UK), the maize starch allowed to settle and 1 ml of the supernatant, estimated to contain 6.7×10^7 to 6.7×10^9 colony-forming units of *B. breve* BBG-001, administered daily. The interventions were started as soon as practicable after randomisation, whether or not enteral feeding had begun, and were continued until 36 weeks' postmenstrual age.

Feeding and clinical care including withholding of the intervention was at the discretion of local clinicians.

Stools at 14 days' postnatal and 36 weeks' postmenstrual age were cultured for *B. breve* using a selective medium provided by the manufacturer. If enough stool was available, the 14-day sample was additionally analysed using a strain-specific quantitative real-time polymerase chain reaction.

Main outcome measures

Primary outcomes

1. Any baby with an episode of bloodstream infection, with any organism other than a skin commensal, more than 72 hours after birth and before 46 weeks' postmenstrual age.
2. Any baby with an episode of NEC Bell stage ≥ 2 .
3. Death before discharge from hospital.

Secondary outcomes

Secondary outcomes included the composite of the primary outcomes, a range of microbiological outcomes including antimicrobial usage and stool colonisation with *B. breve* and antibiotic-resistant pathogens, time to full enteral feeding, weight gain to 36 weeks' postmenstrual age and major neonatal morbidities.

Statistical power

At a two-sided significance level of 5%, a trial of 1300 infants would have 90% power to detect a 40% relative risk (RR) reduction from 15% to 9.1% for each of the primary outcomes. If the outcomes were less frequent, then the trial would have 90% power to detect a 44% RR reduction from 12% to 6.7% or from 10% to 5.6%.

Results

Recruitment continued for 37 months from July 2010; 654 babies were allocated to receive probiotic and 661 placebo. Consent to use data was withdrawn for five babies, and 650 infants in the probiotic group and 660 in the placebo group were included in the intention-to-treat analysis.

Baseline characteristics were well balanced: the overall median gestation age was 28 weeks (48% < 28 weeks); the median birthweight was 1010 g (49% < 1000 g); 91% were exposed to antenatal corticosteroid; 36% were exposed to maternal antibiotics within 24 hours of birth; 53% were delivered by caesarean section; 25% were recruited in the first 24 hours after birth; and the intervention was started at a median age of 44 hours. At 14 days, 96% of those infants who were still alive had received some maternal breast milk, augmented in 48.5% with either donor breast or formula milk.

All comparative analyses were adjusted for sex, gestational age and randomisation within 24 hours of birth. Allowance was made for correlations between multiple births. The primary analysis by intention to treat showed no evidence of benefit for any of the primary outcomes {sepsis: 11.2% vs. 11.7% [adjusted RR 0.97, 95% confidence interval (CI) 0.73 to 1.29]; NEC Bell stage ≥ 2 : 9.4% vs. 10.0% [adjusted RR 0.93, 95% CI 0.68 to 1.27]; and death: 8.3% vs. 8.5% [adjusted RR 0.93, 95% CI 0.67 to 1.30]}.

Of those surviving at 2 weeks' postnatal age, *B. breve* colonisation status was available for 1186 (94%). In total, 724 (61%) infants were positive: 85% of the active intervention group and 37% of the placebo group.

Subgroup analyses by colonisation status, sex, gestational age as per minimisation, birthweight (≥ 1000 or < 1000 g), birth (< 28 weeks' gestational age or ≥ 28 weeks' gestational age) and randomisation within 24 hours of birth suggested reduced sepsis rates in those born at 28 or 29 weeks [odds ratio (OR) 0.39, 95% CI 0.16 to 0.96], but no other differences for any of the primary outcomes.

Secondary outcomes

There were no differences between the groups for secondary outcomes (apart from *B. breve* colonisation) including the composite outcome of late-onset sepsis, NEC or death. One or both of the stool samples collected from 38 out of 611 (6.2%) infants in the probiotic group and 35 out of 619 (5.7%) in the placebo group were colonised with antibiotic-resistant bacteria.

In the probiotic group, colonisation was more likely with each week of increasing gestation (OR 1.36; $p < 0.0001$) and less likely in those given any antibiotic between days 6 and 14 (OR 0.26; $p = 0.0027$).

In a secondary non-random analysis, among those with *B. breve* colonisation status known at 2 weeks, there were trends towards fewer babies with primary outcomes associated with colonisation, but none of the findings was statistically significant.

The interventions were well tolerated; there were no positive cultures of *B. breve* from any normally sterile site and no adverse events related to the interventions were reported.

Throughout the recruitment period, the number of viable organisms in the intervention declined slowly, but remained in the expected range and no contaminants were detected.

Conclusions

We believe that the population recruited into this trial is representative of the total population in this geographic area at risk of NEC and late-onset sepsis. It is the first completed trial performed in accordance with the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use – Good Clinical Practice standard and to have systematically studied stool colonisation in all trial centres. The trial has adequate statistical power and the use of a product containing a single bacterial strain provides a clear result.

Although confirming the short-term safety of probiotic interventions, this trial provides no evidence that this particular product is associated with advantage in this population of babies. This result supports the view that it is necessary to assess the efficacy of different probiotic strains in different clinical situations and challenges the validity of combining trials using different probiotic interventions in meta-analyses.

Implications for clinical practice

The results of this trial provide no evidence that supplementation with *B. breve* BBG-001 would affect the risk of late-onset sepsis, NEC or death in this population.

Implications for research

We find no evidence that further trials should be undertaken of this probiotic in this population.

The results of this trial have implications for the design of trials of other probiotic interventions:

- Colonisation rates of both the active and placebo groups should be monitored throughout the trial.
- Cluster design should be considered to reduce any confounding effects of cross-colonisation.

Future work recommendations

The increasing understanding of the pathogenesis of NEC and late-onset sepsis will inform the choice of probiotics for testing and better define the target population. Future Phase III trials should incorporate monitoring of the quality and viability of the intervention and colonisation rates of participants; cluster design should be considered.

Trial registration

This trial is registered as ISRCTN05511098 and EudraCT 2006-003445-17.

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