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Prebiotics to prevent necrotising enterocolitis in very preterm or very low birth weight infants (Protocol)

Sharif S, Oddie SJ, Heath PT, McGuire W

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[Intervention Protocol]

Prebiotics to prevent necrotising enterocolitis in very preterm or very low birth weight infants

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ABSTRACT

Objectives

This is a protocol for a Cochrane Review (intervention). The objectives are as follows:

To evaluate the effectiveness of enteral supplementation with prebiotic oligosaccharides on the risk of necrotising enterocolitis, and associated morbidity and mortality, in very preterm or very low birth weight infants.

BACKGROUND

This review will assess the trial evidence for the effectiveness of enteral supplementation with prebiotic oligosaccharides for preventing necrotising enterocolitis (NEC) in very preterm or very low birth weight (VLBW) infants. Other Cochrane Reviews assess the evidence for prebiotics in combination with probiotics ('synbiotics') or probiotics alone (Sharif 2020; Sharif 2021).

Description of the condition

Necrotising enterocolitis is a syndrome of acute intestinal necrosis which affects about one in 20 very preterm (born before 32 weeks' gestation) or VLBW (birth weight less than 1500 g) infants (Horbar 2012). The risk factors for NEC include being extremely preterm (born before 28 weeks' gestation) or extremely low birth weight (ELBW; birth weight less than 1000 g), and intrauterine growth restriction or compromise indicated by absent or reversed end-diastolic flow velocities (AREDFV) in antenatal Doppler studies of the umbilical artery (Samuels 2017). Infants who develop NEC experience more episodes of severe infection, have lower levels of nutrient intake, grow more slowly, and have longer durations of hospital stay than gestation-comparable infants who do not (Battersby 2018; Berrington 2012). The associated mortality rate is about 20%, and, in infants who survive NEC — especially if it is associated with bloodstream infections — there is a high risk of neurodevelopmental problems and disabilities (Hickey 2018).

The pathogenesis of NEC is incompletely understood, but intestinal dysbiosis, infection and inflammation are thought to contribute (Eaton 2017; Mara 2018; Stewart 2016). Evidence exists that the pattern, diversity and stability of the intestinal microbiome (microbial life and genes) is associated with the risk of developing NEC (Masi 2019; Olm 2019; Stewart 2012; Warner 2016). Feeding with human milk compared with cow-milk formula reduces the risk of NEC in very preterm or VLBW infants (Cleminson 2015; Quigley 2019). One putative mechanism for this protective effect is that 'prebiotic' oligosaccharides, which are abundant in human milk (but not in standard formula), promote the growth of non-pathogenic probiotic micro-organisms, such as lactobacilli and bifidobacteria. These modulate the intestinal microbiome and enhance mucosal barrier functions (Embleton 2017; Granger 2020; Walsh 2019). Compared with human milk-fed term infants, however, very preterm or VLBW infants tend to harbour fewer intestinal probiotic micro-organisms, and more potential pathogens, which might be due to the dysbiotic effects of antibiotic exposure and enteral fasting during the early neonatal period (Stewart 2017).

Description of the intervention

Prebiotics are a diverse family of complex glycans (chains of polymerised carbohydrates) that promote intestinal colonisation by probiotic micro-organisms (Davani-Davari 2019; Gibson 2017). Human milk contains numerous prebiotic substances, predominantly galacto-oligosaccharides and fructo-oligosaccharides (based on the sugars galactose and fructose, respectively), that influence the intestinal microbiome in preterm infants (Boehm 2008; Nolan 2020). More than 150 different prebiotic oligosaccharides have been detected in human milk, with about 20 of these accounting for almost all human milk oligosaccharide content in most women. The pattern of human milk oligosaccharides produced varies markedly between individual women, and can vary temporally (depending on the stage of lactation) within individual woman (Austin 2019; Smilowitz 2013).

Newborn infants do not digest human milk oligosaccharides. Rather, these are primarily nutrient sources for intestinal probiotic micro-organisms, particularly bifidobacteria (Alcon-Giner 2020; Jost 2015). Emerging evidence suggests that specific human-milk oligosaccharides can promote probiotic predominance and reduce intestinal dysbiosis in very preterm infants (Masi 2020; Underwood 2015). Manufactured or plant-based (for example, inulin) prebiotic oligosaccharides are less heterogeneous than natural human-milk oligosaccharides, typically consisting of chains of galactose or fructose, usually with a terminal glucose monomer (Johnson-Henry 2016). These include lactulose, a non-absorbable disaccharide synthesized from galactose and fructose (MacGillivray 1959). Evidence exists that giving supplemental, synthetic, prebiotic oligosaccharides to formula-fed very preterm infants stimulates the growth of an intestinal microflora that is similar to that found in infants fed with maternal milk (Autran 2018; Boehm 2008; Kapiki 2007; Veereman-Wauters 2011). Prebiotic oligosaccharides are added as ingredients to some cow-milk formulas for feeding newborn infants for whom sufficient human milk is not available (Salminen 2020). Studies using animal models, however, have not provided consistent evidence of efficacy in preventing NEC-like syndromes (Nolan 2020).

How the intervention might work

The principal mechanism of action of supplemental prebiotics is likely to be the enhancement of probiotic micro-organism growth and intestinal colonisation (Nolan 2020; Underwood 2019). Probiotic bacteria and fungi use prebiotic oligosaccharides as a major source of nutrients (Alcon-Giner 2020). Promoting a probiotic-rich intestinal microbiome is thought to benefit infants via several mechanisms. Probiotics may out-compete pathogens for nutrients. Bifidobacteria and lactobacilli ferment prebiotic oligosaccharides to produce short-chain fatty acids, including lactic acid, butyric acid, and propionic acid, that inhibit adhesion of pathogenic bacteria and modulate intestinal epithelial development, integrity, and barrier function (Johnson-Henry 2016; Zmora 2018). Short-chain fatty acids also lower the pH level of the stool and may enhance intestinal motility, thereby improving feed tolerance (Armanian 2019). Other putative actions include stimulating differentiation and proliferation of enterocytes (cells of the intestinal lining), enhancing expression of intestinal digestive enzymes, and improving intestinal mucosal barrier integrity (Johnson-Henry 2016; Sanders 2019).

While there is some trial-based evidence that enteral administration of exogenous probiotics reduces the risk of NEC and associated mortality and morbidity in very preterm or VLBW infants, concerns exist that effect size estimates are inflated by publication bias (Sharif 2020). Another major barrier to use of probiotic supplementation is uncertainty about the optimal constitution of products, as well as availability, and regulatory and licensing issues (Berrington 2019; Duffield 2019; Fleming 2019; Pell 2019; Vermeulen 2020). Furthermore, although existing data are reassuring with regard to safety, probiotic bacteraemia or fungaemia (the potentially problematic presence of live bacteria/fungi in the bloodstream) and other adverse effects have been reported in preterm infants (Bertelli 2015; Esaiassen 2016; Zbinden 2015).

Why it is important to do this review

Necrotising enterocolitis and its associated complications — particularly invasive infection — are the commonest causes of mortality and serious morbidity beyond the early neonatal period in very preterm or VLBW infants (Berrington 2012). It is plausible that prebiotic supplementation might promote endogenous probiotic growth and colonisation, and reduce the risk of NEC and its associated morbidity and mortality (with fewer risks than exogenous probiotic supplementation). Appraising and synthesising the trial evidence about the effectiveness and safety of prebiotic supplementation could inform practice, policy and research.

OBJECTIVES

To evaluate the effectiveness of enteral supplementation with prebiotic oligosaccharides on the risk of necrotising enterocolitis, and associated morbidity and mortality, in very preterm or very low birth weight infants.

METHODS

Criteria for considering studies for this review

Types of studies

We will include randomised controlled trials (RCTs) or quasi-RCTs (including cluster-RCTs). We will exclude cross-over studies.

Types of participants

We will include very preterm (< 32 weeks' gestation) or VLBW (< 1500 g) infants.

Types of interventions

Enteral prebiotics: any combination or dose of prebiotic oligosaccharides (galacto-oligosaccharides (GOS); fructo-oligosaccharides (FOS); inulin; or lactulose), commenced within 14 days of birth and continued (at least) daily for (at least) one week versus placebo or no prebiotic.

We will not include trials of synbiotics (combinations of probiotics and prebiotics), or trials of other substances that may have some prebiotic properties, for example lactoferrin. The effectiveness of these interventions is addressed in other Cochrane Reviews (Pammi 2020; Sharif 2021).

Types of outcome measures

We will assess effects on infant- and family-important outcomes, principally neonatal morbidities that plausibly affect rates of mortality or neurodisability. We will not include surrogate outcomes such as stool colonisation patterns.

Primary outcomes

- NEC before 44 weeks' post-menstrual age or discharge from hospital confirmed at surgery or autopsy or diagnosed using standardised clinical and radiological criteria (VON 2020), specifically:
  - at least one of: bilious gastric aspirate or emesis; or abdominal distention; or blood in stool; *and*
  - at least one of: abdominal radiograph showing pneumatosis intestinalis; or gas in the portal venous system; or free air in the abdomen.
- All-cause mortality before 44 weeks' post-menstrual age or discharge from hospital.

Secondary outcomes

- Late-onset (> 48 hours after birth) invasive infection occurring before 44 weeks' post-menstrual age or discharge from hospital, confirmed by culture of bacteria or fungi from blood or cerebrospinal fluid or from a normally sterile body space
- Duration of hospitalisation from birth in surviving infants
- Neurodevelopmental impairment assessed by a validated test after 12 months' post-term, to include: neurological evaluations, developmental scores, and classifications of disability, including cerebral palsy and auditory and visual impairment

Search methods for identification of studies

We will use the criteria and standard methods of Cochrane Neonatal.

Electronic searches

We will search the Cochrane Central Register of Controlled Trials (CENTRAL latest issue), Ovid MEDLINE (1946 onwards), OVID Embase (1974 onwards), OVID Maternity & Infant Care Database (1971 onwards), and the Cumulative Index to Nursing and Allied Health Literature (1982 onwards) using a combination of text words and MeSH terms described in Appendix 1. We will limit the search outputs with the relevant search filters for clinical trials as recommended in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2020). We will not apply any language restrictions.

We will search clinical trials registries for ongoing or recently completed trials (clinicaltrials.gov; the World Health Organization's International Trial Registry and Platform, and the ISRCTN Registry).

Searching other resources

We will examine the reference lists of any articles selected for inclusion in this review.

Data collection and analysis

We will use the standard methods of Cochrane Neonatal.

Selection of studies

Two review authors (SS, PTH or SJO) will independently screen the title and abstract of all studies and then assess the full articles for all potentially relevant trials. We will exclude those studies that do not meet all the inclusion criteria, and we will state the reason(s) for exclusion. We will discuss any disagreements until consensus is achieved, with referral to WM for final decision, if necessary.

Data extraction and management

Two authors (SS, PTH, SJO or WM) will extract data independently, using a form to aid extraction of information on design, methodology, participants, interventions, outcomes and treatment effects from each included study. We will discuss any disagreements until we reach a consensus. If data from the study

reports are insufficient, we will contact the report authors for further information.

Assessment of risk of bias in included studies

Independently, two review authors (SS, PTH, SJO or WM) will assess the risk of bias (low, high or unclear) of all included trials using the Cochrane risk of bias tool (Higgins 2011), for these domains:

- sequence generation (selection bias);
- allocation concealment (selection bias);
- blinding of participants and personnel (performance bias);
- blinding of outcome assessment (detection bias);
- incomplete outcome data (attrition bias);
- selective reporting (reporting bias);
- any other bias (including baseline imbalance).

We will resolve disagreements through discussion or by involving a third assessor. See Appendix 2 for a description of risk of bias for each domain.

Measures of treatment effect

We will analyse the treatment effects in the individual trials and report risk ratio (RR) and risk difference (RD) for dichotomous data and mean difference (MD) for continuous data, with respective 95% confidence intervals (CI). We will determine the number needed to treat for an additional beneficial outcome (NNTB) or an additional harmful outcome (NNTH) for analyses with a statistically significant difference in the RD.

Unit of analysis issues

The unit of analysis will be the participating infant in individually randomised trials and the neonatal unit (or sub-unit) for cluster-randomised trials. For cluster-randomised trials, we will undertake analyses at the level of the individual while accounting for the clustering in the data using the methods recommended in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2020).

Dealing with missing data

Where feasible, we intend to carry out analysis on an intention-to-treat basis for all outcomes. Whenever possible, we will analyse all participants in the treatment group to which they were randomised, regardless of the actual treatment received. If we identify important missing data (in the outcomes) or unclear data, we will request the missing data by contacting the original investigators. We will make explicit the assumptions of any methods used to deal with missing data. We may perform sensitivity analyses to assess how sensitive results are to reasonable changes in the undertaken assumptions. We will address the potential impact of missing data on the findings of the review in the 'Discussion' section.

Assessment of heterogeneity

We will examine the treatment effects of individual trials and heterogeneity between trial results by inspecting the forest plots. We will calculate the I^2 statistic for each analysis to quantify inconsistency across studies and describe the percentage of variability in effect estimates that may be due to heterogeneity rather than to sampling error. If we detect high levels of heterogeneity ($I^2 > 75\%$), we will explore the possible sources (for example, differences in participants or interventions).

Assessment of reporting biases

If at least 10 trials are included in a meta-analysis, we will examine a funnel plot for asymmetry visually and with Harbord's modification of Egger's test (Harbord 2006).

Data synthesis

We will use a fixed-effect model inverse variance meta-analysis for combining data where trials examined the same intervention and the populations and methods of the trials were judged to be similar.

Subgroup analysis and investigation of heterogeneity

When high heterogeneity is detected ($I^2 > 75\%$), we plan to examine the potential causes in subgroup analyses for the primary outcomes, specifically:

- type of prebiotic: GOS; FOS; inulin; or lactulose;
- type of enteral feeding permitted for participating infants: human milk; formula; both.

Sensitivity analysis

We will undertake sensitivity meta-analyses to explore effects on primary outcomes in trials:

- in which most participants were ELBW or extremely preterm;
- in which participants were infants with intrauterine growth restriction or AREDFV;
- at low risk of bias (removing data from trials at uncertain risk or high risk of bias).

Summary of findings and assessment of the certainty of the evidence

We will use the GRADE approach, as outlined in the GRADE Handbook (Schünemann 2013), to assess the certainty of evidence of the following (clinically relevant) outcomes: NEC, all-cause mortality, late-onset infection and neurodevelopmental impairment.

Independently, two authors (SS, PTH, SJO or WM) will assess the certainty of the evidence for each of the outcomes above. We will consider evidence from RCTs as high certainty, but downgrade the evidence one level for serious (or two levels for very serious) limitations based upon the following: design (risk of bias), consistency across studies, directness of the evidence, precision of estimates, and presence of publication bias (Walsh 2021).

The GRADE approach results in an assessment of the certainty of a body of evidence as one of four grades.

- High: we are very confident that the true effect lies close to that of the estimate of the effect.
- Moderate: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
- Low: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.

- Very low: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

We will use the GRADEpro GDT Guideline Development Tool to create a summary of findings table to report the certainty of the evidence.

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Jeffrey Horbar and Steven Kwasi Korang have peer reviewed and offered feedback on this protocol.

The methods section of the protocol is based on a standard template used by Cochrane Neonatal.

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APPENDICES

Appendix 1. Electronic search strategy

Indicative strategy developed and tested for Cochrane Register of Controlled Trials (CENTRAL)

[To be adapted for MEDLINE, Embase, Maternity & Infant Care Database (MIDIRS), and CINAHL Plus]

#1 MeSH descriptor: [Probiotics] explode all trees

#2 (probiotic*):ti,ab,kw (Word variations have been searched)

#3 MeSH descriptor: [Bifidobacterium] explode all trees

#4 (bifidobacterium*):ti,ab,kw (Word variations have been searched)

#5 MeSH descriptor: [Lactobacillus] explode all trees

#6 (lactobacill*):ti,ab,kw (Word variations have been searched)

#7 MeSH descriptor: [undefined] explode all trees

#8 MeSH descriptor: [Saccharomyces boulardii] this term only

#9 (Saccharomyces):ti,ab,kw (Word variations have been searched)

#10 #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9

#11 MeSH descriptor: [Prebiotics] explode all trees

#12 (prebiotic*):ti,ab,kw (Word variations have been searched)

#13 MeSH descriptor: [Oligosaccharides] explode all trees

#14 (oligosaccharide*):ti,ab,kw (Word variations have been searched)

#15 MeSH descriptor: [Inulin] explode all trees

#16 (inulin*):ti,ab,kw (Word variations have been searched)

#17 ((fructooligosaccharide* or fructo-oligosaccharide* or FOS or FOSs or galacto-oligosaccharide* or galactooligosaccharide*)):ti,ab,kw (Word variations have been searched)

#18 MeSH descriptor: [Lactoferrin] explode all trees

#19 (lactoferrin*):ti,ab,kw (Word variations have been searched)

#20 MeSH descriptor: [Lactulose] explode all trees

#21 (lactulose*):ti,ab,kw

#22 #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19 or #20 or #21

#23 MeSH descriptor: [Synbiotics] explode all trees

#24 (synbiotic*):ti,ab,kw (Word variations have been searched)

#25 (((probiotic* and prebiotic*) NEAR/4 combin*)):ti,ab,kw (Word variations have been searched)

#26 #23 OR #24 OR #25

#27 #10 OR #22 OR #26

#28 MeSH descriptor: [Infant, Newborn] explode all trees

#29 MeSH descriptor: [Premature Birth] explode all trees

#30 neonat*:ti,ab,kw (Word variations have been searched)

#31 neo-nat*:ti,ab,kw (Word variations have been searched)

#32 newborn or new born* or newly born*:ti,ab,kw (Word variations have been searched)

#33 preterm or preterms or (pre term) or (pre terms):ti,ab,kw (Word variations have been searched)

#34 preemie* or premie or premies:ti,ab,kw (Word variations have been searched)

#35 prematur* near/3 (birth* or born or deliver*):ti,ab,kw (Word variations have been searched)

#36 low near/3 (birthweight* or birth weight*):ti,ab,kw (Word variations have been searched)

#37 lbw or vlbw or elbw:ti,ab,kw (Word variations have been searched)

#38 infan* or baby or babies:ti,ab,kw (Word variations have been searched)

#39 #28 or #29 or #30 or #31 or #32 or #33 or #34 or #35 or #36 or #37 or #38

#40 #27 AND #39

Appendix 2. Risk of bias tool

Sequence generation (checking for possible selection bias). Was the allocation sequence adequately generated?

For each included study, we will categorise the method used to generate the allocation sequence as:

- low risk (any truly random process, e.g. random number table; computer random number generator);
- high risk (any non-random process, e.g. odd or even date of birth; hospital or clinic record number); or
- unclear risk.

Allocation concealment (checking for possible selection bias). Was allocation adequately concealed?

For each included study, we will categorise the method used to conceal the allocation sequence as:

- low risk (e.g. telephone or central randomisation; consecutively numbered sealed opaque envelopes);
- high risk (open random allocation; unsealed or non-opaque envelopes, alternation; date of birth); or
- unclear risk.

Blinding of participants and personnel (checking for possible performance bias). Was knowledge of the allocated intervention adequately prevented during the study?

For each included study, we will categorise the methods used to blind study participants and personnel from knowledge of which intervention a participant received. Blinding will be assessed separately for different outcomes or class of outcomes. We will categorise the methods as:

- low risk, high risk or unclear risk for participants; and
- low risk, high risk or unclear risk for personnel.

Blinding of outcome assessment (checking for possible detection bias). Was knowledge of the allocated intervention adequately prevented at the time of outcome assessment?

For each included study, we will categorise the methods used to blind outcome assessment. Blinding will be assessed separately for different outcomes or class of outcomes. We will categorise the methods as:

- low risk for outcome assessors;
- high risk for outcome assessors; or
- unclear risk for outcome assessors.

Incomplete outcome data (checking for possible attrition bias through withdrawals, dropouts, protocol deviations). Were incomplete outcome data adequately addressed?

For each included study and for each outcome, we will describe the completeness of data including attrition and exclusions from the analysis. We will note whether attrition and exclusions were reported, the numbers included in the analysis at each stage (compared with the total randomised participants), reasons for attrition or exclusion where reported, and whether missing data were balanced across

groups or were related to outcomes. Where sufficient information was reported or supplied by the trial authors, we will re-include missing data in the analyses. We will categorise the methods as:

- low risk (< 20% missing data);
- high risk (≥ 20% missing data); or
- unclear risk.

Selective reporting bias. Are reports of the study free of suggestion of selective outcome reporting?

For each included study, we will describe how we investigated the possibility of selective outcome reporting bias and what we found. For studies in which study protocols were published in advance, we will compare prespecified outcomes versus outcomes eventually reported in the published results. If the study protocol was not published in advance, we will contact study authors to gain access to the study protocol. We will assess the methods as:

- low risk (where it is clear that all of the study's prespecified outcomes and all expected outcomes of interest to the review have been reported);
- high risk (where not all the study's prespecified outcomes have been reported; one or more reported primary outcomes were not prespecified outcomes of interest and are reported incompletely and so cannot be used; study fails to include results of a key outcome that would have been expected to have been reported); or
- unclear risk.

Other sources of bias. Was the study apparently free of other problems that could put it at a high risk of bias?

For each included study, we will describe any important concerns we had about other possible sources of bias (for example, whether there was a potential source of bias related to the specific study design or whether the trial was stopped early due to some data-dependent process). We will assess whether each study was free of other problems that could put it at risk of bias as:

- low risk;
- high risk;
- unclear risk.

If needed, we will explore the impact of the level of bias through undertaking sensitivity analyses.

CONTRIBUTIONS OF AUTHORS

All authors (WM, SS, SJO, PTH) contributed to the development of the protocol.

DECLARATIONS OF INTEREST

WM has no conflict of interest to declare.

SS has no conflict of interest to declare.

SJO has no conflict of interest to declare.

PTH works as a Consultant Paediatrician (Infectious diseases), St Georges University Hospitals NHS Trust, UK.

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