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Best practice guidelines

Addressing safety concerns of probiotic use in preterm babies

ABSTRACT

More than 10,000 preterm babies worldwide have been enrolled in trials evaluating probiotics administration for the prevention of necrotising enterocolitis, with very few adverse events reported. Despite this, probiotic safety is frequently cited as a concern when using this intervention. This review addresses why a preterm baby may be at risk when administered a live microbial product, short- and longer-term safety data in relation to probiotic use and regulatory aspects around probiotic manufacture and preparations.

1. Introduction

Over the last 25 years, multiple studies have evaluated the role of probiotic administration in preterm babies for the prevention of necrotising enterocolitis (NEC) and late onset sepsis (LOS). Probiotics are defined as 'live micro-organisms which when given in sufficient amounts confer health benefit on the host' [1]. Much debate about safety has occurred around using probiotics in this vulnerable patient population [2]. The 2014 Cochrane Database of Systematic Reviews (CDSR) evaluated 24 randomised controlled trials (RCTs) of probiotic administration and, based on 20 studies in 5529 preterm babies < 37 weeks gestation and < 2500 g birthweight, concluded that probiotic use in preterm babies was both safe and effective [3] based on reductions in NEC, without systemic infection with the supplemented organism(s) within the reported trials. This review also noted that data available to make these conclusions were substantial in comparison with other neonatal interventions including surfactant administration, therapeutic hypothermia and resuscitation in room air. However, probiotic evaluation in large RCTs outside of the preterm population has not always been associated with safe outcomes [4]. It is also true that 'absolute safety' cannot be proved within even very large trials and will always require ongoing surveillance and clinician awareness that rare events may not be detected. In 2018, The European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN), published a strain specific systematic review and network meta-analysis of probiotic use in preterm babies and were unable to identify the optimal strain, dose or combination of probiotics to reduce NEC. It concluded that clinicians are left using inadequately tested, potentially unsafe and possibly ineffective treatments [5].

This review discusses why preterm babies might be at risk from administration of live microbial products; short-term problems associated with probiotic administration; outcomes of longer term follow-up studies of preterm babies who have been enrolled in probiotic trials; and the factors associated with regulation and production of probiotic products.

2. The intestinal barrier

At the time of birth, preterm babies' intestinal barrier must undergo rapid maturation and closure. The intestinal barrier comprises four layers; the microbiological, chemical (mucous), physical and immunological layers. In preterm babies, many differences exist in each of these layers.

It is generally accepted that the preterm microbiological layer differs to that of the term infant or adult and harbours more potentially pathogenic bacteria [6]. This is one of the factors that may predispose the preterm baby to conditions like NEC or LOS. The chemical (or mucous) layer which ordinarily prevents intestinal bacteria interacting with intestinal enterocytes, is also thought to be somewhat deficient with MUC2 messenger RNA (mRNA) [the structural component of the mucous layer] only reaching adult levels at around 27 weeks' gestation [7], and being easily disrupted by events and therapies associated with preterm birth. The physical layer of the intestinal barrier is provided by intestinal enterocytes (IECs). This layer is a selectively permeable barrier which is designed to allow the uptake of food and nutrients whilst at the same time excluding potentially harmful microbes and antigens. Permeability across the IEC barrier has previously been shown to be increased in preterm babies [8]. Furthermore, other important cellular components of the physical layer (namely Paneth cells) are also known to be reduced in number which limits the production of antimicrobial proteins [9]. Finally, the preterm immunological layer is developmentally different to that of term infants. Previous studies have shown that in particular, the preterm intestine harbours more Toll like receptor 4 (TLR-4) receptors, acting as gut growth regulators in the foetus, but which when stimulated with bacterial products such as lipopolysaccharide (LPS) ex-utero can result in a poorly controlled downstream pro-inflammatory immunological response ultimately leading to production of the powerful pro-inflammatory cytokine interleukin 8 (IL8) [10].

Bacterial translocation is defined as the passage of bacteria or antigens through the intestinal barrier into a normally sterile site [11]. Although bacterial translocation has been poorly studied in the preterm baby, in paediatric studies, the gut has been suggested as one of the main origins of sepsis and organ dysfunction in critically unwell children [12], and significant proportions of neonatal septicaemias are with 'gut-derived' organisms, suggesting an aetiological role for translocation in LOS [13]. In addition changes in the gut microbiome have been associated with the development of LOS [14].

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Coupled with developmental immaturity of the intestinal barrier in preterm babies, inflammatory conditions like NEC or LOS may further weaken the intestinal barrier and predispose the infant to uncontrolled transfer of bacteria and antigens across the intestinal barrier. Careful consideration of the risk-benefit ratio is essential when conducting studies that administer large quantities of a live microbial product such as probiotics to patients with an ineffective or damaged intestinal barrier [15].

3. Short term concerns regarding probiotic administration to preterm babies

In the 2014 CDSR, Alfaleh evaluated 24 trials of probiotic administration for the prevention of NEC, LOS and death in preterm babies, noting that none of the studies reported systemic infection associated with probiotic use.

The majority of reported short-term adverse consequences associated with probiotic administration are limited to individual case reports of bacteraemias. These have been reported in the literature in association with administration of both *Bifidobacteria* and *Lactobacilli* containing probiotic products [16–23]. Among these reports, a number of babies developed or had underlying intestinal pathologies but mortality from probiotic bacteraemia did not occur. Probiotics are usually sensitive to β-lactam antibiotics [24] which tend to be the most widely used antibiotics in neonatal practice [25,26]. These individual reports represent a small number of babies in the context of the much larger numbers that are included in recent meta-analyses [27].

Probiotic organisms are fastidious anaerobes and are difficult to grow using standard culture media [28], such that bacteraemias from probiotic micro-organisms may be under-reported and under-recognised. Given the potential risk for probiotic associated bacteraemias in preterm babies, centres evaluating or routinely using probiotics must know the specific type and strain(s), know and monitor the sensitivity patterns of these probiotic strains and be confident that their microbiology laboratories can accurately identify these organisms during evaluations for suspected LOS. It would also be prudent to ensure that 'empirical' antibiotic choice for LOS covers the probiotic strain(s). These safety measures and assurances should be in place before routinely implementing probiotic therapy [2].

4. Neurodevelopmental follow-up studies of babies enrolled to probiotic trials

Follow-up of babies enrolled in probiotic studies has two advantages. The first provides assurances on the longer-term safety aspects of probiotic administration to this vulnerable population. The second addresses whether or not other later benefits might be associated with probiotic use outside the immediate postnatal period.

Probiotics could potentially improve neurodevelopmental outcomes by reducing the incidence of NEC or LOS [29]. Alternatively, they could affect the gut-brain-microbiome axis through modulation of intestinal dysbiosis which itself has been linked to a number of mood and behavioural disorders [30], and through this mechanism improve neurodevelopmental outcomes with or without measurable impact on NEC or LOS.

Four follow-up studies of preterm babies randomised in probiotic trials have reported 'longer-term' follow-up outcomes, from 12 months to 5 years of age. Chou and colleagues [31] assessed 301 babies born weighing < 1500 g at age 3 years using the Bayley Scales of Infant and Toddler Development II [32], who had previously been enrolled in a trial evaluating the probiotic combination of *Bifidobacteria infantis* and *Lactobacillus acidophilus*. The group reported no differences in the incidence of mortality or neurodevelopmental impairment between probiotic and control groups (45/153 (29.4%) vs 49/148 (33.1%), $p = 0.1$). Sari and colleagues performed a follow-up study of 242 children born < 33 weeks' gestation or < 1500 g and enrolled to a trial comparing *Lactobacillus sporogenes* with placebo. They also reported no difference in incidence of survival without major neurodevelopmental impairment (100/121 (82.6%) vs 98/121 (81.0%), $p = 0.92$) [33]. One further study enrolled 249 infants < 2500 g and < 37 weeks' gestation at birth and reported similar rates of suboptimal scores at 12 months corrected age using the Hammersmith Infant Neurological assessment [34].

The largest long-term follow-up study was performed by investigators of the Australasian ProPrems trial. This trial recruited 1099 babies born < 32 weeks' gestation and weighing < 1500 g with participants randomised to the probiotics combination *Bifidobacterium infantis*, *Bifidobacterium lactis* and *Streptococcus thermophilus* or placebo. The trial reported a 54% reduction in the secondary outcome of NEC in babies receiving the probiotics combination. Outcome data for 735 (67%) ProPrems trial participants were available at age 2–5 years with survival free from major neurosensory impairment comparable between children who received probiotics and those who received placebo (281 (75.3%) vs 271 (74.9%); RR 0.98 (95%CI 0.76, 1.26); $p = 0.88$) [29].

Although many more babies have been enrolled to probiotics trials than have been assessed in follow-up, published data suggests no significant evidence of adverse outcomes among those babies who have been evaluated in longer-term outcome studies, although also somewhat disappointingly, no apparent benefit.

5. Probiotic products: quality and safety

The majority of commercially available probiotics are classified and used as food or dietary supplements, not medicines. As such they are not subject to the same stringent regulatory processes as a therapeutic intervention or medicine. There are no universally agreed frameworks for probiotic regulation. In Europe, they are regulated by the European Food Standard's Agency (EFSA) which has published a list of microbial cultures that have a 'Qualified Presumption of Safety' and do not therefore require safety assessments. In the United States they are regulated by the Food and Drug Administration (FDA) but usually classified as foods or dietary supplements and, whilst their production is required to comply with Good Manufacturing Practice (GMP) guidelines, these guidelines do not extend to testing quality or efficacy [35,36].

This lower level of regulatory scrutiny can impact on both the quality and safety of probiotic products. This is evidenced in the study by Lewis and colleagues who found that the contents of many bifidobacterial probiotic products differ from the ingredients list and that only 1 of 16 probiotics perfectly matched its bifidobacterial label claims [37]. One further factor with regards to product contents, rarely considered, is that during the probiotic manufacturing process, dead bacteria and their fragments cannot be separated from the live bacteria, therefore the final product will contain a mixture of dead bacteria, bacterial fragments and live bacteria. Whilst probiotic labels usually report the number of live bacterial colony forming units in a product, information regarding the other bacterial components is less clear. However, dead bacteria and bacterial components are also capable of instigating an immune response which may be relevant in immunocompromised patients [35].

Product contamination by pathogenic organisms is also a recognised risk. This is especially pertinent to the preterm baby population following the death of an infant which was attributed to a Rhizopus oryzae contaminated probiotic product [38]. To protect high risk patients, it is recommended that probiotics should meet stringent microbiological standards and that product testing results should be available before administering probiotic products to at-risk patients [2].

For centres using probiotics in preterm babies, continuous and stringent in-house quality and safety monitoring of probiotic preparations is essential. Until high-quality pharmaceutical-grade probiotic preparations (single dose packaging, stable strains, cross-tested against contaminants) for the vulnerable preterm population are available (due

late 2019; personal communication SEJ), consideration must be given to analysing each new batch of probiotics for both the presence and quantification of probiotic organisms by polymerase chain reaction, as well as for purity by standard microbiological culture techniques.

6. Conclusion

Although there are no international consensus guidelines governing quality and safety of probiotic products, they are generally considered safe for consumption by the general population. However, caution is advised in vulnerable patient groups, including preterm babies. The preterm intestinal barrier is not as well developed as that of the term infant or older child and may be weakened further during episodes of LOS. This predisposes the preterm baby to bacterial translocation. Short-term adverse effects encountered with probiotic use in this population are limited to individual case reports of bacteraemia. In the context of the large numbers of babies randomised in probiotic trials, these reports are few and balancing the potential benefits versus risks of probiotic administration is up to individual clinicians and/or neonatal units. For those centres that proceed with administering probiotics to preterm babies, these organisms may be difficult to grow in the laboratory so adequate capacity to confidently identify and isolate the bacteria at local level is essential. In addition, centres using probiotics must undertake regular quality testing of the products used. Long-term follow-up of babies previously enrolled to probiotic RCTs is limited, but there is no reported evidence of either harm or of any major impact on longer-term neurodevelopmental outcomes.

Declaration of Competing Interest

None declared.

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