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ORIGINAL ARTICLE

Multi-modal approach to prophylaxis of necrotizing enterocolitis: clinical report and review of literature

G. Schmolzer · B. Urlesberger · Michaela Haim · J. Kutschera · G. Pichler · E. Ritschl · B. Resch · F. Reiterer · W. Müller

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Abstract For the first time a multimodal approach to NEC prophylaxis is reported, consisting of early trophic feeding with human breast milk, and enteral administration of an antibiotic, an antifungal agent, and probiotics. A retrospective analysis of local protocol of NEC prophylaxis is presented. Included were all VLBWI admitted to the NICU, including transfers within the first 28 days of life. These infants were divided into two groups, an ''inborn group'' (infants admitted within the first 24 h of life) and an ''outborn group'' (infants admitted after the onset of their second day of life). Prophylaxis of NEC according to protocol was started at the day of admission, and was continued until discharge. Between 1998 and 2004, 405 VLBWI were admitted, including all transfers within the first 28 days of life. A total of 334 (82%) infants were admitted within the first 24 h of life (inborn group), and 71 (18%) were admitted after 24 h of life (outborn group). Five infants developed clinical features of necrotizing enterocolitis. The inborn group showed a NEC incidence of 0.7% (two infants), whereas the outborn group showed a NEC incidence of 4.5% (three infants), respectively. This difference was significant (P=0.049, Fisher's exact test). A surgical treatment with bowel resection was performed in two infants (both from the outborn group). The present study used a combination of different strategies, all having shown to have some beneficial effect, but not having brought a clinical breakthrough in single administration studies. Combinated were the beneficial effects of human breast milk feeding, oral antiobiotics, oral antifungal agents, and the administration of probiotics. In a homogenous group of preterm infants, using this protocol of multimodal NEC prophylaxis, there was a very low incidence of NEC, when started within the first 24 h of life.

G. Schmolzer (✉) · B. Urlesberger · M. Haim
J. Kutschera · G. Pichler · E. Ritschl · B. Resch
F. Reiterer · W. Müller
Division of Neonatology, Department of Pediatrics,
Medical University of Graz, Auenbruggerplatz 30,
8036 Graz, Austria
e-mail: georg.schmoelzer@meduni-graz.at

Introduction

Necrotizing enterocolitis (NEC) is the most common life-threatening gastrointestinal disease in neonates [68]. Despite three decades of research efforts, NEC remains a major cause of death for neonates undergoing surgery. The incidence of NEC has increased in the past decades, as the advantages in neonatology and the modern neonatal intensive care unit have led to the increased survival of infants of even smaller birth weight and younger gestational age [30]. Additionally, as surfactant has become standard of care in preterm infants, the number of very low birth weight (VLBW) infants at risk for developing NEC has continued to rise [29, 30, 50].

NEC occurs in one to three in 1,000 live births [37, 52], equally often in female and male [52]. NEC most commonly affects babies born between 30 and 32 weeks and is often diagnosed during the second week of life [36, 38]. The mortality from NEC has been cited as 10–50% of all affected infants [29]. The surgical mortality has decreased over the last several decades from 70% to numbers between 20 and 50% [29].

NEC has a multifactorial etiology and the pathogenesis has not fully been elucidated. The classic histological finding is coagulation necrosis present in over 90% of specimens [3]. This finding suggests the importance of ischemia in the pathogenesis of NEC [29]. Inflammation and bacterial overgrowth are also present [3]. There is an assumption that NEC occurs by the interaction of three events: Initially a mucosal injury occurs due to intestinal ischemia, followed by inflammation of the disturbed mucosal integrity with subsequent necrosis of the affected area. The further steps are colonization by pathogenic bacteria and excess protein substrate in the intestinal lumen. Furthermore the immunologic immaturity of the neonatal gut has been implicated in the development of NEC [37].

NEC affects most commonly the terminal ileum, caecum and ascending colon. Typical clinical signs include abdominal distension, bile- or blood stained emesis or gastric aspirate, abdominal wall erythema and bloody stools. Diagnosis is based on radiographic evidence as bowel distension, ileus, pneumatosis intestinalis or bowel perforation. Management includes parenteral nutrition and antibiotics, or surgical approach with bowel resection. Over the last years, different strategies for prevention of NEC have been developed. None of the strategies has been really a break through. This article presents a multi-modal approach of a prevention strategy combining well-known strategies to one concept including enteral administration of antibiotics, antifungal agent, probiotics, plus early trophic feeding with human breast milk, resulting in a low NEC incidence within a neonatal intensive care unit. Furthermore, it gives an overview of different NEC prevention strategies.

Methods

We performed a retrospective analysis of a local protocol of NEC prophylaxis. Included were all VLBW infants admitted to the neonatal intensive care unit (tertiary center), including transfers within the first 28 days of life. Two groups of infants were analyzed: patients, who were admitted on the first day of life formed the ''inborn group'', all patients, who were admitted after the onset of their second day of life formed the ''outborn group''. In inborn group prophylaxis of NEC according to protocol started within the first 24 h of life and continued to discharge, in outborn group prophylaxis of NEC according to protocol started after admission and was continued to discharge. Whereas inborn group represents our standard patient collective; the outborn group serves as a comparison group, having had no standardized NEC prophylaxis before admission. Being a third level universitarian neonatal intensive care unit, preterm infants have to be admitted from peripheral hospitals, if problems occurred during their stay in those units. None of those units uses a NEC-Prophylaxis protocol similar to the present.

The protocol of NEC prophylaxis consists of enteral antibiotics, enteral antifungal agent, enteral probiotics, and trophic feeding as follows: Gentamycin (7 mg/kg 12 hourly per os), Nystatin (10.000 IU/kg 6 hourly per os), and enteral probiotics (*Lactobacillus Rhamnosus* GG 1 g=1×10^9 colony forming units per day, divided in two doses).

The feeding protocol at the NICU starts with the administration of pooled donor human milk (1 ml/kg all 3 h) on the first day of life, with a stepwise increase of 1 ml/kg per day during the first week of life, with a change to expressed breast milk of the preterm infant's mother. If no expressed breast milk of the preterm infant's mother was available, feeding with pooled donor human milk is continued until the infant's nutrition reaches fully enteral feeding. The aim is to reach fully enteral nutrition within the 14th day of life. Mother milk is pasteurized until the infant reaches 32 weeks of gestation or a body weight of 1,500 g.

All infants received a prophylactic administration of Indomethacin for 3 days, according to a previously published protocol [45, 46].

Various classifications have been published for NEC [5, 8, 70]. In the present study NEC was defined, using Bell's criteria stage II or greater [5].

Results

Over a 7-year period from 1998 to 2004, 405 very-low-birth-weight infants have been admitted to the NICU, including transfers within the first 28 days of life.

Out of these 405 infants, 334 (82%) infants have been admitted within the first 24 h of life (inborn group), and 71 (18%) have been admitted after 24 h of life (outborn group). Patient's demographical data and the incidence of NEC are shown in Table 1. There is no difference between birth weight and gestational age in the two studied groups.

Five of the 347 surviving and studied infants developed clinical features of NEC. The inborn group showed a NEC incidence of 0.7% (two infants), whereas the outborn group showed a NEC incidence of 4.5% (three infants). This difference was significant (P=0.049, Fisher's exact test).

Table 1 Patients' demographical data and NEC incidence

	Inborn group	Outborn group
n	334	71
Birth weight (median, min/max)	1,040 (383/1,500) g	1,110 (478/1,500) g
<751 g	22% (74)	15% (11)
751–1,000 g	25% (82)	15% (11)
1,001–1,250 g	28% (93)	34% (24)
1,251–1,500 g	25% (85)	35% (25)
Gestational weeks, completed (median, min/max)	28 (23/37) weeks	30 (23/33) weeks
Ventilated infants	95% (318)	49% (35)
Days on ventilator (median, min/max)	12 (1/114)	21.5 (2/153)
Death	16.2% (54)	5.6% (4)
Incidence of NEC in survivors	0.7% (2)	4.5% (3)*
NEC-OP	0	2

Prophylaxis of NEC started at admission in both groups, inborn group (prophylaxis of NEC started within the first 24 h of life) and outborn group (prophylaxis of NEC started after admission, between 2nd and 28th day of life), and was continued to discharge

*P=0.049 (Fisher's exact test)

Whereas the two NEC patients of the inborn group were admitted within the first 24 h of life, the three patients of the outborn group were admitted on 11th, 12th, and 14th day of life, respectively. Two patients of the outborn group already had diagnosis of NEC at admission and both needed surgical treatment. The third patient of the outborn group had an additionally diagnosis of phenylketonuria and therefore had to be fed differently, according to special protocol (including only small amounts of human milk). The latter patient (of outborn group) plus the two patient of the inborn group did not need a surgical intervention, they were treated with parenteral nutrition and administration of antibiotics.

Discussion

In the present study the incidence of NEC was 0.7%, when this group of patients was submitted to a standardized protocol of NEC prophylaxis, starting from the first day of life (inborn group). Shimura et al. [63] described a similar low incidence of NEC (0.6%), Lin et al. [40] described a low incidence of NEC (1.1%) in a group of patients receiving probiotics. In contrast, the incidence of NEC in Canada, USA and most other western countries reaches 5–7% [3, 27, 30, 31, 33, 60].

The infants of the outborn group cannot represent a normal control group, of course. This group was analyzed separately, not having had NEC prophylaxis according to protocol starting on first day of life. None of these infants had received NEC prophylaxis before admission. Within this outborn group incidence of NEC was according to the literature, out of this perspective the outborn group was called ''control group''. Two of the three patients with NEC within this group had already been diagnosed with NEC before admission. The third patient was admitted to our NICU with an additionally diagnosis of phenylketonuria. Because of this metabolic disease the patient had to be fed according to a special protocol, allowing only small amounts of human milk. Therefore this patient was the only one, who did not receive NEC prophylaxis according to standardized protocol. The patient developed NEC after 28 days of life (during stay within our NICU).

Additionally to NEC prophylaxis protocol all infants received a prophylactic administration of indomethacin for the first 3 days to prevent intracranial hemorrhage, whereas only some of the outborn group received indomethacin. In the early 1980s the use of indomethacin has been implicated, followed by randomized controlled trials [19–21, 23] of prophylactic administration. Although these trials reported effectiveness of indomethacin administration in preventing IVH and PDA, unexpected side effects secondary to decreased splanchicus blood flow resulted in a restrain from a universal recommendation of the use of indomethacin [19]. Controversially, O'Donovan et al. [35], in a retrospective study, and Cooke et al. [13] for the Cochrane Neonatal Collaborative Review Group, concluded that prophylactic indomethacin treatment was not associated with an increased risk for the development of NEC.

Over the last years different strategies for prevention of NEC, such as changing the feeding practice, using donor breast milk, probiotics and immunoglobins have been developed. The following paragraphs summarize these different NEC prevention strategies published in the literature. However, in contrast to the multi modal approach of the present study, most of these studies used a single approach only.

Feeding practices

The GI tract is an active organ in utero. The fetus swallows amniotic fluid composed of nutrients, growth factors, and immunoglobulins [51]. Low gestational age reflects the developmental immaturity of the intestine. On the other hand, the intestine is ready to digest enteral nutrition and to tolerate bacteria and other organisms acquired after birth.

Thus, the question of fast versus slow and early versus delayed feeding has been discussed extensively in the literature, several randomized trails have shown no effect on the incidence of NEC [54] so far.

Delaying the initiation of feeds has been shown to postpone the onset of NEC. Therefore, it was a common practice to withhold feeds from premature infants especially after the initiation of parenteral nutrition. However, this practice is not without a risk: already short term starving periods in animals showed mucosal atrophy and increased permeability of the gut mucosa [57]. Although, the role of enteral feeding as a risk factor for NEC has been emphasized, 5–10% of NEC occurs in babies who have never been fed enterally [41]. Small trophic feeds have been shown to stimulate maturation of GI function, although they have not been shown to decrease the incidence of NEC [41]. Brown and Sweet [9] postulated that an aggressive enteral feeding protocol of more than 20 ml/kg/day increases the incidence of NEC. Bersteh et al. [7], randomly assigned infants to have feeding volumes increased daily by 20 ml/kg rather than being held at minimal volume for the first 10 days of life. The study stopped when seven infants in the group with advancing feeding volumes developed NEC, compared to one infant in the minimal feeding group. Thus the authors concluded, that advancing feeding volumes increased the risk of NEC.

Breast milk

Breast milk is the recommended source of enteral nutrition for preterm infants and has been demonstrated to decrease the incidence of NEC [6, 44]. Several epidemiological and animals studies indicated that breast milk is protective. Formula fed newborn infants have a six- to tenfold increase of NEC when compared with innate breast milk fed infants [41]. Therefore, it would seem prudent to consider minimal feeding volumes of breast milk, when available, rather than formula for the first 7–10 days of life. Multiple factors in breast milk are hypothesized to prevent the development of NEC, including immunoglobulins, erythropoietin, IL-10, epidermal growth factor (EGF) and platelet-activating factor (PAF)-acetylhydrolase [54].

EGF, a potent protein that produces a variety of biologic responses such as enhanced proliferation and differentiation of epithelial cells. It has been reported as an important trophic factor for the developing intestine [48]. Reduced level of salivary EGF has been identified in neonates at the time of onset of NEC compared with age-matched control neonates [64]. Breast milk, including colostrum is a major source of EGF but formula is exclusive EGF [34]. The enteral administration of recombinant EGF reduced the development and the incidence of NEC in a neonatal rat model [15].

PAF, a potent phospholipid inflammatory mediator produced by inflammatory cells, endothelial cells, platelets, and bacteria of the intestinal flora has been implicated in the pathogenesis of NEC [54]. In infants developing NEC [11, 12], elevated PAF levels and decreased levels of PAF-acetylhydrolase (PAF-AH), the enzyme responsible for the degradation of PAF, have been reported. In animal experiments using neonatal rats, an injection of PAF directly into the aorta caused an intestinal disease similar to NEC, whereas the administration of PAF-AH prevent the development of NEC in the same model [22]. Thus, the presence of PAF-AH in breast milk may contribute to its protective effect.

These findings suggest that breast milk may be the recommended source of enteral nutrition for preterm infants. The additional beneficial effect of breast milk is the delivery of immunoprotective factors to the immature gut mucosa [41].

Donor breast milk versus formula milk

When expressed breast milk of the preterm infant's mother is not available, an alternative is banked milk from donor mothers. However, donor human milk is typically the breast milk of mothers who have delivered at term. This milk has a lower content of protein and host defence protein compared to breast milk of a mother who has delivered a preterm infant [44]. Several studies of comparison between Donor human breast milk versus formula milk in premature infants have been published [24, 25, 39, 43, 44, 53, 61, 67, 69, 71, 73]. Mc Guire and Anthony [44] summarized four small trials (Gross 1983, Lucas 1990, Svenningsen 1982 and Tyson 1983), all initiated 20 years ago. Aim of the trails was to compare the feeding of Donor human milk versus formula for preventing NEC in preterm infants. None of the trials found a statistically significant difference between human milk versus formula in regard to the incidence of NEC. Although, none of the single studies showed a significant result, the meta-analysis of all studies did show a significant reduced relative risk of NEC with feeding of Donor human milk. Furthermore, NEC was three times less likely, and confirmed NEC was four times less likely, in infants who received donor breast milk rather than formula milk [44]. However, other authors have failed to reproduce the data; they failed to show a reduced risk in the incidence of NEC by using donor human milk. Recently,

Schanler et al. [61] compared preterm formula and donor human milk as substitutes for mother's own milk in premature infants. They reported similar rates of NEC and late onset sepsis within the groups.

Probiotics

The term probiotic was derived from the Greek, meaning ''for life''. An expert panel commissioned by the Food and Agriculture Organisation of the United Nations (FAO) and the World Health Organisation (WHO) defined probiotics as ''Live microorganisms which when administered in adequate amounts confer a health benefit on the host.'' This is the definition that should be used, and probiotics should not be referred to as biotherapeutic agents [55]. The range of effects of probiotics on the gut are wide and include changes of intestinal permeability, enhanced mucosal IgA response, increases in the production of anti-inflammatory cytokines and protection of the mucosa against colonization from pathogens [49].

The intestinal microflora in VLBW infants may be dominated by many pathogens such as *Enterococcus faecalis*, *Escherichia coli*, *Staphylococcus epidermidis*, *Enterobacter cloacae*, *Klebsiella pneumonia*, and *Staphylococcus haemolyticus* [28, 55]. Several studies have investigated the intestinal microflora of infants with NEC. Clostridium perfringens has been isolated from 40% of infants with necrotizing entercolitis, compared with 13% of controls [55]. Bell et al. [28] described increased numbers of gram-negative bacteria (in particular *Escherichia coli* and *Klebsiella*) in the stools of infants with NEC.

Based on these observation and previously published data from animal studies, it has been postulated that probiotics may offer similar protection against NEC in premature infants. Several studies have used different strains of probiotics and different administration regimes (length of treatment, dosage) in preterm infants. None of the trials has reported adverse effects, furthermore, there was not observed any episode of pathogenic infection caused by a probiotic organism [1, 4, 14, 32, 40].

A successful colonization rate of 80–90% for *Lactobacillus GG* has been reported in previous published studies. In contrast, Agarwal et al. [1] showed colonization about 25% in VLBW infants with *Lactobacillus GG* and suggested that colonization depends on the interplay of multiple factors in the intestinal milieu. For *Lactobacillus acidophilus* a successful colonization rate of 60–86% has been reported. Hoyos et al. [32] showed a 60% decrease of NEC in neonates during 1 year in a Columbian NICU using two strains of probiotic *Lactobacillus acidophilus* and *Bifidobacterium infantis*. They reported a threefold decrease of NEC cases and a fourfold decrease in NEC mortality; however, the comparison was with historical controls. In a prospective randomized controlled trial, Lin et al. [40] reported a significantly lower incidence of NEC in a group of preterm infants receiving probiotics (1.1%), versus the control group (5.4%).

Larger clinical trials are necessary to evaluate the safetyness and efficacy of this promising intervention, to better define both the benefits and the risks for premature infants. Recently, Schultz et al. [62] described a possible further direction for the administration of probiotics. They showed that the temporary colonization of an infant with *L. GG* may be possible by colonizing the pregnant mother before delivery.

Immunoglobulins

A number of reports have been published, which suggest that orally taken immunoglobulins (IgA and IgG) have an immunoprotective effect on the gastrointestinal mucosa [16, 59]. Premature infants have decreased levels of immunoglobulins, especially secretory IgA [17]. In a randomized clinical trial, Eibl et al. [16] evaluated the efficacy of an oral immunoglobulin preparation (73% IgA and 26% IgG) in reducing the incidence of NEC in infants of low birth weight for whom breast milk from their mothers was not available. They reported no cases of NEC in the treatment group of 88 infants compared with six cases of NEC in the control group of 91 infants, respectively. Rubaltelli et al. [59] evaluated in a randomized clinical trial the efficacy of an oral immunoglobulin preparation (containing monomeric IgG in a concentration of 90%) in reducing the incidence of NEC in infants of VLBW for whom maternal breast milk was not available. They reported not any case of NEC in the treatment group of 65 infants compared with four cases of NEC in the control group of 67 infants, respectively. However, other authors have failed to reproduce the data, they failed to show a decrease in the incidence of NEC by using oral immunoglobulins. In a prospective randomized trial, Fast et al. [17] compared the efficacy of oral gentamycin versus oral IgA–IgG for the prophylaxis of NEC. NEC was diagnosed in 13 cases in the oral IgA–IgG group of 100 infants compared with one case in the oral gentamycin group of 100 infants. Richter et al. [56] examined the efficacy of oral IgG prophylaxis for the prevention of NEC compared to a historical cohort group; they reported no difference in the incidence in both groups and concluded that infants were not protected against NEC by the use of oral IgG.

For the Cochrane Neonatal Collaborative Review Group, Foster and Cole [18] recently concluded that based on the available trials, the evidence does not support the administration of oral immunoglobulin for the prevention of NEC.

Oral antibiotics

Published data suggest that the use of enteral antibiotics may be effective as NEC prophylaxis. Grylack and Scanlon [26] evaluated the effects of prophylactic oral gentamycin therapy in the prevention of NEC. In their study, none of the 20 gentamycin treated infants developed NEC, whereas four (of 22 infants) within the control group. In contrast, Rowley and Dahlenburg [58] reported no decrease in the incidence of NEC using an oral gentamycin regimen. Recently, in a prospective, double blind, randomized, placebo controlled study, Siu et al. [66] evaluated the effectiveness of oral vancomycin in the prophylaxis of NEC. They reported a NEC incidence of 13% (9 of 71) in the group of infants receiving oral vancomycin, compared to a NEC incidence of 28% (19 of 69) in the group receiving the placebo solution. For the Cochrane Neonatal Collaborative Review Group, Bury and Tudehope [10], evaluated five trials where oral antibiotics were used as prophylaxis against NEC in low birth weight and preterm infants. Their analysis suggests that oral administration of prophylactic enteral antibiotics results in a statistically significant reduction of NEC and in NEC-related deaths.

In a recently published article, Bell [4] summarizes different prevention strategies. Calculating the numbers needed to treat for the different strategies to prevent NEC, the most effective strategy was the administration of enteral antibiotics, followed by human breast milk feeding. However, the risks of enteral antibiotics have not been quantified yet, thus this strategy has never been widely adopted, due to concerns about the emergence of resistant bacteria and absorption of antibiotics from the gut [4]. However, such adverse effects have not been reported so far.

Oral antifungal agents

Mucocutaneus candidiasis (oral, perineal, other skin sites) is a frequent finding in the neonatal unit (3.2% of all admissions Gupta 1996, 7.8% Faix 1989) [2]. Oral nystatin is the most commonly used non-absorbable agent, followed by oral miconazole, which is also non-absorbable and an alternative to nystatin. Oral or intravenous fluconazole has been used both in the treatment of systemic infections [2], and more recently to reduce fungal colonization and infection [42].

For the Cochrane Neonatal Collaborative Review Group, Austin and Darlow [2] evaluated three trials, where oral antifungal agents were used as prophylaxis against systemic candida infection in preterm infants. They concluded that based on the available trials, the evidence does not yet support the oral administration of antifungal agents. In one of the analyzed trails, Sims et al. [65] reported a statistically significant reduction in the incidence of systemic fungal infection. Additionally, in none of these studies adverse effects were reported [2].

Conclusion

For the first time, a multimodal approach to NEC prophylaxis is reported. The present study used a combination of different strategies, all having shown to have some beneficial effect, but not having brought a clinical breakthrough in single administration studies. To prevent NEC effectively, the beneficial effects of human breast milk feeding, oral antibiotics, oral antifungal agents, and the administration of probiotics were combined together. In a homogenous group of preterm infants, using this protocol of multimodal NEC prophylaxis, the NEC incidence was as low as 0.7%, when prophylactic strategy was started within the first 24 h of life. This incidence was significantly lower when compared to a control group of infants without a primary prophylaxis strategy.

References

1. Agarwal R, Sharma N, Chaudhry R, Deorari A, Paul VK, Gewolb IH, Panigrahi P (2006) Effects of oral lactobacillus GG on enteric microflora in low-birth-weight neonates. J Pediatr Gastroenterol Nutr 36:397–402
2. Austin NC, Darlow B (2004) Prophylactic oral antifungal agents to prevent systemic candida infection in preterm infants. Cochrane Database Syst Rev (1):CD003478
3. Ballance WA, Dahms BB, Shenker N, Kliegman RM (1990) Pathology of neonatal necrotizing enterocolitis: a ten-year experience. J Pediatr 117(1 Pt 2):S6–S13
4. Bell EF (2005) Preventing necrotizing enterocolitis: what works and how safe? Pediatrics 115(1):173–174
5. Bell MJ, Ternberg JL, Feigin RD, Keating JP, Marshall R, Barton L, Brotherton T (1978) Neonatal necrotizing enterocolitis. Therapeutic decisions based upon clinical staging. Ann Surg 187(1):1–7
6. Berseth CL (2005) Feeding strategies and necrotizing enterocolitis. Curr Opin Pediatr 17(2):170–173
7. Berseth CL, Bisquera JA, Paje VU (2003) Prolonging small feeding volumes early in life decreases the incidence of necrotizing enterocolitis in very low birth weight infants. Pediatrics 111(3):529–534

8. British Association for Perinatal Paediatrics and the Public Health Laboratory Service Communicable Disease Surveillance Centre (1983) Surveillance of necrotising enterocolitis, 1981–82. Br Med J 287:824–826
9. Brown EG, Sweet AY (1978) Preventing necrotizing enterocolitis in neonates. JAMA 240:2452–2454
10. Bury RG, Tudehope D (2001) Enteral antibiotics for preventing necrotizing enterocolitis in low birthweight or preterm infants. Cochrane Database Syst Rev (1):CD000405
11. Caplan MS, Sun XM, Hseuh W, Hageman JR (1990) Role of platelet activating factor and tumor necrosis factor-alpha in neonatal necrotizing enterocolitis. J Pediatr 116(6):960–964
12. Caplan MS, Lickerman M, Adler L, Dietsch GN, Yu A (1997) The role of recombinant platelet-activating factor acetylhydrolase in a neonatal rat model of necrotizing enterocolitis. Pediatr Res 42(6):779–783
13. Cooke L, Steer P, Woodgate P (2003) Indomethacin for asymptomatic patent ductus arteriosus in preterm infants (Review). Cochrane Database Syst Rev (2):CD003745
14. Dani C, Biadaioli R, Bertini G, Martelli E, Rubaltelli FF (2002) Probiotics feeding in prevention of urinary tract infection, bacterial sepsis and necrotizing enterocolitis in preterm infants: a prospective double-blind study. Biol Neonate 82(2):103–108
15. Dvorak B, Halpern MD, Holubec H, Williams CS, McWilliam DL, Dominguez JA, Stepankova R, Payne CM, McCuskey RS (2002) Epidermal growth factor reduces the development of necrotizing enterocolitis in a neonatal rat model. Am J Physiol Gastrointest Liver Physiol 282(1):G156–G164
16. Eibl MM, Wolf HM, Furnkranz H, Rosenkranz A (1988) Prevention of necrotizing enterocolitis in low-birth-weight infants by IgA–IgG feeding. N Engl J Med 319(1):1–7
17. Fast C, Rosegger H (1994) Necrotizing enterocolitis prophylaxis: oral antibiotics and lyophilized enterobacteria vs oral immunoglobulins. Acta Paediatr Suppl 396:86–90
18. Foster J, Cole M (2004) Oral immunoglobulin for preventing necrotizing enterocolitis in preterm and low birth-weight neonates. Cochrane Database Syst Rev (1):CD001816
19. Fowlie PW (1996) Prophylactic indomethacin: systematic review and meta-analysis (Review). Arch Dis Child Fetal Neonatal Ed 74(2):F81–F87
20. Fowlie PW, Davis PG (2002) Prophylactic intravenous indomethacin for preventing mortality and morbidity in preterm infants (Review). Cochrane Database Syst Rev (3):CD000174
21. Fowlie PW, Davis PG (2003) Prophylactic indomethacin for preterm infants: a systematic review and meta-analysis (Review). Arch Dis Child Fetal Neonatal Ed 88(6): F464–F466
22. Furukawa M, Lee EL, Johnston JM (1993) Platelet-activating factor-induced ischemic bowel necrosis: the effect of platelet-activating factor acetylhydrolase. Pediatr Res 34(2):237–241
23. Grosfeld JL, Chaet M, Molinari F, Engle W, Engum SA, West KW, Rescorla FJ, Scherer LR III (1996) Increased risk of necrotizing enterocolitis in premature infants with patent ductus arteriosus treated with indomethacin. Ann Surg 224(3):350–355
24. Gross SJ (1983) Growth and biochemical response of preterm infants fed human milk or modified infant formula. N Engl J Med 308(5):237–241
25. Gross SJ, David RJ, Bauman L, Tomarelli RM (1980) Nutritional composition of milk produced by mothers delivering preterm. J Pediatr 96(4):641–644
26. Grylack LJ, Scanlon JW (1978) Oral gentamicin therapy in the prevention of neonatal necrotizing enterocolitis. A controlled double-blind trial. Am J Dis Child 132(12):1192–1194
27. Guthrie SO, Gordon PV, Thomas V, Thorp JA, Peabody J, Clark RH (2003) Necrotizing enterocolitis among neonates in the United States. J Perinatol 23(4):278–285
28. Hammerman C, Bin-Nun A, Kaplan M (2004) Germ warfare: probiotics in defense of the premature gut (Review). Clin Perinatol 31(3):489–500
29. Henry MC, Moss RL (2004) Current issues in the management of necrotizing enterocolitis (Review). Semin Perinatol 28(3):221–233
30. Holman RC, Stoll BJ, Clarke MJ, Glass RI (1997) The epidemiology of necrotizing enterocolitis infant mortality in the United States. Am J Public Health 87(12):2026–2031
31. Horbar JD, Badger GJ, Lewit EM, Rogowski J, Shiono PH (1997) Hospital and patient characteristics associated with variation in 28-day mortality for very low birth weight infants. Pediatrics 99:149–156
32. Hoyos AB (1999) Reduced incidence of necrotizing enterocolitis associated with enteral administration of *Lactobacillus acidophilus* and *Bifidobacterium infantis* to neonates in an intensive care unit. Int J Infec Dis 3:197–202
33. Hsueh W, Caplan MS, Qu XW, Tan XD, De Plaen IG, Gonzalez-Crussi F (2003) Neonatal necrotizing enterocolitis: clinical considerations and pathogenetic concepts. Pediatr Dev Pathol 6(1):6–23. Epub 2002 Nov 11 (Review)
34. Iacopetta BJ, Grieu F, Horisberger M, Sunahara GI (1992) Epidermal growth factor in human and bovine milk. Acta Paediatr 81(4):287–291
35. Kamitsuka MD, Horton MK, Williams MA (2000) The incidence of necrotizing enterocolitis after introducing standardized feeding schedules for infants between 1,250 and 2,500 g and less than 35 weeks of gestation. Pediatrics 105(2):379–384
36. Kliegman RM, Fanaroff AA (1981) Neonatal necrotizing enterocolitis: a nine-year experience. Am J Dis Child 135(7):603–607
37. Kosloske AM (1984) Pathogenesis and prevention of necrotizing enterocolitis: a hypothesis based on personal observation and a review of the literature (Review). Pediatrics 74(6):1086–1092
38. Ladd AP, Rescorla FJ, West KW, Scherer LR III, Engum SA, Grosfeld JL (1998) Long-term follow-up after bowel resection for necrotizing enterocolitis: factors affecting outcome. J Pediatr Surg 33(7):967–972
39. Lindemann PC, Foshaugen I, Lindemann R (2004) Characteristics of breast milk and serology of women donating breast milk to a milk bank. Arch Dis Child Fetal Neonatal Ed 89(5):F440–F441
40. Lin HC, Su BH, Chen AC, Lin TW, Tsai CH, Yeh TF, Oh William (2005) Oral probiotics reduce the incidence and severity of necrotizing enterocolitis in very low birth weight infants. Pediatrics 115(1):1–4
41. Lucas A, Cole TJ (1990) Breast milk and neonatal necrotising enterocolitis. Lancet 336(8730):1519–1523
42. Manzoni P, Maestri A, Gomirato G, Takagi H, Watanabe D, Matsui S (2005) Prophylactic fluconazole is effective in preventing fungal colonization and fungal systemic infections in preterm neonates: a single-center, 6-year, retrospective cohort study. Pediatrics
43. McGuire W, Anthony MY (2001) Formula milk versus preterm human milk for feeding preterm or low birth weight infants (Review). Cochrane Database Syst Rev (3):CD002972

44. McGuire W, Anthony MY (2003) Donor human milk versus formula for preventing necrotising enterocolitis in preterm infants: systematic review. Arch Dis Child Fetal Neonatal Ed 88:F11–F14
45. Ment LR, Oh W, Ehrenkranz RA, Phillip AG, Vohr B, Allan W, Makuch RW, Taylor KJ, Schneider KC, Katz KH et al (1994) Low-dose indomethacin therapy and extension of intraventricular hemorrhage: a multicenter randomized trial. J Pediatr 124(6):951–955
46. Ment LR, Oh W, Ehrenkranz RA, Philip AG, Vohr B, Allan W, Duncan CC, Scott DT, Taylor KJ, Katz KH et al (1994) Low-dose indomethacin and prevention of intraventricular hemorrhage: a multicenter randomized trial. Pediatrics 93(4):543–550
47. Mercado MC, Brodsky NL, McGuire MK, Hurt H (2004) Extended interval dosing of gentamicin in preterm infants. Am J Perinatol 21(2):73–77
48. Miettinen PJ, Berger JE, Meneses J, Phung Y, Pedersen RA, Werb Z, Derynck R (1995) Epithelial immaturity and multiorgan failure in mice lacking epidermal growth factor receptor. Nature 376(6538):337–341
49. Millar M, Wilks M, Costeloe K (2003) Probiotics for preterm infants? Arch Dis Child Fetal Neonatal Ed 88:F354–F358
50. Nadler EP, Upperman JS, Ford HR (2001) Controversies in the management of necrotizing enterocolitis. Surg Infect (Larchmt) Summer 2(2):113–119; discussion 119–120 (Review)
51. Neu J (1996) Necrotizing enterocolitis: the search of an unifying pathogenic theory leading to prevention. Pediatr Clin North Am 43:409–432
52. Noerr B (2003) Current controversies in the understanding of necrotizing enterocolitis. Part 1 (Review). Adv Neonatal Care 3(3):107–120
53. Raiha NC, Heinonen K, Rassin DK, Gaull GE (1976) Milk protein quantity and quality in low-birthweight infants: I. Metabolic responses and effects on growth. Pediatrics 57(5):659–684
54. Reber KM, Nankervis CA (2004) Necrotizing enterocolitis: preventative strategies (Review). Clin Perinatol 31(1):157–167
55. Reid G, Jass J, Sebulsky MT, McCormick JK (2003) Potential uses of probiotics in clinical practice (Review). Clin Microbiol Rev 16(4):658–672
56. Richter D, Bartmann P, Pohlandt F (1998) Prevention of necrotizing enterocolitis in extremely low birth weight infants by IgG feeding? Eur J Pediatr 157(11):924–925
57. Rothmann D, Udall JN, Pang KY, Kirkham SE, Walker WA (1985) The effect of short-term starvation in the newborn rabbit. Pediatr Res 19:727–731
58. Rowley MP, Dahlenburg GW (1978) Gentamicin in prophylaxis of neonatal necrotising enterocolitis. Lancet 2(8088):532
59. Rubaltelli FF, Benini F, Sala M (1991) Prevention of necrotizing enterocolitis in neonates at risk by oral administration of monomeric IgG. Dev Pharmacol Ther 17(3–4):138–143
60. Sankaran K, Puckett B, Lee DSC, Seshia M, Boulton J, Qiu Z, Lee SK, The Canadian Neonatal Network (2004) Variations in incidence of necrotizing enterocolitis in canadian neonatal intensive care units. J Pediatr Gastroenterol Nutr 39:366–372
61. Schanler RJ, Lau C, Hurst NM, Smith EO (2005) Randomized trial of donor human milk versus preterm formula as substitutes for mothers' own milk in the feeding of extremely premature infants. Pediatrics 116(2):400–406
62. Schultz M, Gottl C, Young RJ, Iwen P, Vanderhoof JA (2004) Administration of oral probiotic bacteria to pregnant women causes temporary infantile colonization (Review). J Pediatr Gastroenterol Nutr 38:293–297
63. Shimura K (1990) Necrotizing enterocolitis? A Japanese survey. NICU 3:5–7
64. Shin CE, Falcone RA Jr, Stuart L, Erwin CR, Warner BW (2000) Diminished epidermal growth factor levels in infants with necrotizing enterocolitis. J Pediatr Surg 35(2):173–176; discussion 177
65. Sims ME, Yoo Y, You H, Salminen C, Walther FJ (1988) Prophylactic oral nystatin and fungal infections in very-low-birthweight infants. Am J Perinatol 5(1):33–36
66. Siu YK, Ng PC, Fung SC, Lee CH, Wong MY, Fok TF, So KW, Cheung KL, Wong W, Cheng AF (1998) Double blind, randomised, placebo controlled study of oral vancomycin in prevention of necrotising enterocolitis in preterm, very low birthweight infants. Arch Dis Child Fetal Neonatal Ed 79(2):F105–F109
67. Stein H, Cohen D, Herman AA, Rissik J, Ellis U, Bolton K, Pettifor J, MacDougall L (1986) Pooled pasteurized breast milk and untreated own mother's milk in the feeding of very low birth weight babies: a randomized controlled trial. J Pediatr Gastroenterol Nutr 5(2):242–247
68. Stoll B (1994) Epidemiology of necrotizing enterocolitis. Clin Perinatol 21:205–219
69. Tyson JE, Lasky RE, Mize CE, Richards CJ, Blair-Smith N, Whyte R, Beer AE (1983) Growth, metabolic response, and development in very-low-birth-weight infants fed banked human milk or enriched formula. I. Neonatal findings. J Pediatr 103(1):95–104
70. Walsh MC, Kliegman RM (1986) Necrotizing enterocolitis: treatment based on staging criteria (Review). Pediatr Clin North Am 33(1):179–201
71. Wight NE (2001) Donor human milk for preterm infants. J Perinatol 21(4):249–254
72. Wight NE (2003) Donor human milk versus formula for preventing necrotising enterocolitis in preterm infants: systematic review. J Pediatr 143(1):137–138
73. Wight NE (2005) Donor milk: down but not out. Pediatrics. 116(6):1610; author reply 1610–1611