TITLE: INHIBITION OF HMGB1 IMPROVES NECROTIZING ENTEROCOLITIS BY INHIBITING NLRP3 VIA TLR4 AND NF-KB SIGNALING PATHWAYS

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CONTENT:

Necrotizing enterocolitis (NEC) is an acquired gastrointestinal disease which primarily affects premature babies. It is the most common gastrointestinal emergency in the newborn and may lead to death in severely affected infants. Although the exact cause is unknown, the critical elements are thought to be prematurity, enteral feeding, an inappropriate pro-inflammatory response and bacterial colonization. The current rodent models of NEC aim at mimicking these factors by feeding animals with lipopolysaccharide (LPS)-supplemented formula milk in combination with repeated exposure to hypoxia and/or hypothermia. There is strong evidence that the initial bacterial colonization of the newborn intestine plays a pivotal role in the development of NEC. NEC is characterized by an extensive hemorrhagic inflammatory necrosis of the distal small bowel and proximal colon with extensive infiltration of neutrophils.

Intestinal epithelial cell lines constitutively express several members of a novel family of transmembrane receptors designated toll-like receptors (TLRs) that may serve as major links between the innate and adaptive mucosal immune responses. TLR4 is the primary receptor needed for the promotion of macrophage activation, cytokine release, and tissue damage. It mediates the recognition of antigens in the intestinal lumen as LPS due to the activation of NF-κB via increasing the production of proinflammatory cytokines. Extracellular high mobility group box 1 (HMGB1) induces inflammatory responses by directly acting on pattern recognition receptors, including TLR2 and 4. HMGB1, a ubiquitous and abundant nuclear protein, can either be passively released into the extracellular milieu in response to necrotic signals or actively secreted in response to inflammatory signals. When releases from cells, HMGB1 can elicit proinflammatory responses in different cell types, such as endothelial cells, macrophages, and neutrophils. Activated NLRP3 couples adaptor protein apoptosis-associated speck like protein (ASC) and caspase-1 to form a multiprotein cytosolic complex, NLRP3 inflammasome. NLRP3 inflammasome plays a crucial role in the innate immune system. Recent studies have shown that the NLRP3 inflammasome is involved in several adaptive immune diseases. Mutations in NLRP3 lead to chronic autoinflammatory syndromes.

Even studies have shown the role of HMGB1 and NLRP3 in animal models of acute colitis has been reported. However, the contribution of HMGB1 to NLRP3 inflammasome activation has not been explored in NEC. In this study, we studied the effect of HMGB1 expression on NLRP3 expression and some inflammatory factor to explore the relationship between HMGB1 and NLRP3 in NEC.

NEC rat models were constructed and treated with HMGB1 inhibitor Glycyrrhizin (GL) with different concentration. Inflammatory condition of intestinal tissue in newborn NEC rats was observed by H&E staining. The mRNA and protein expression of HMGB1, NLRP3, TLR4, NF-κB and Caspase-1 were determined by real-time PCR and western blot, respectively. Content of IL-1β and TNF-α was determined by ELISA assay. Human intestinal epithelial cell lines were induced to NEC by LPS. LPS-induced cells were transfected with siRNA-HMGB1 and NLRP3 plasmid vector. The mRNA and protein expression of HMGB1, NLRP3, TLR4, NF-κB, Caspase-1, IL-1β and TNF-α were determined by real-time PCR and western blot, respectively.

The mRNA and protein expression of HMGB1 and NLRP3 in NEC group was significantly higher than the control group. Inhibition of HMGB1 expression improved intestinal inflammation in newborn NEC rats. The expression of HMGB1, NLRP3, TLR4, NF-κB and Caspase-1 was up-regulated in NEC and was weakened after treating with GL. LPS induction to intestinal epithelial cells markedly increased the expression of HMGB1, NLRP3, TLR4, NF-κB, Caspase-1, IL-1β and TNF-α. Knockdown of HMGB1 abolished the increase of expression, while further transfection with NLRP3 plasmid vector recovered the increase.
HMGB1 and NLRP3 were all up-regulated in the development of NEC. Inhibition on HMGB1 could improve the intestinal inflammation in NEC by inhibiting NLRP3 via TLR4 and NF-κB signaling pathways.

COI: None declared
ID: 132
TITLE: BOVINE COLOSTRUM TO PREPARE OR REPAIR THE INTESTINE FOR FORMULA FEEDING IN PRETERM NEONATES
AUTHORS: Yanqi Li1, Xiaoyu Pan1, Duc Ninh Nguyen1, Shuqiang Ren1, Per Torp Sangild1,2,3
AFFILIATIONS: 1 Comparative Pediatrics and Nutrition, University of Copenhagen, Copenhagen, Denmark; 2 Department of Pediatrics and Adolescent Medicine, Rigshospitalet, Copenhagen, Denmark; 3 Hans Christian Andersen Children’s Hospital, Odense University Hospital and University of Southern Denmark

CONTENT:

Mother’s own milk (MM) improves gut health and protect against necrotizing enterocolitis (NEC) in preterm infants. Due to absence or insufficient supply of mother’s milk, many infants often receive formula before or after MM feeding during the first weeks. It is unknown how such ‘diet shifts’ may affect intestinal health. Bovine colostrum (COL) has been shown to improve gut health and protect against NEC in preterm pigs, relative to infant formula (FOR). Using preterm pigs as a model for preterm infants, and bovine colostrum (COL) to reflect MM, we tested the hypothesis that COL feeding before or after formula (FOR) feeding protects against FOR-induced intestinal impairment.

Seventy-four preterm pigs received increasing volumes of COL or FOR until d 5. At this age, pigs were euthanized or fed either COL or FOR for another 4 d, resulting in six groups: COL or FOR until d 5 (C5, F5, both n=11), COL or FOR until d 9 d (CC, FF n=12-13), COL followed by FOR (CF, n=14) and FOR followed by COL (FC, n=13). Blood and tissues were collected on d 5 and d 9 for measurement of clinical variables together with structural, functional and gene expression parameters in the intestine.

FOR reduced the weight gain and physical activity and increased the NEC incidence (64 vs. 27%) on d 5, relative to COL (F5 vs C5, P<0.05). Intestinal structure and function, measured as villus height and crypt depth, brush border enzymes, hexose absorption, and intestinal integrity, were reduced in F5 vs. C5 pigs, together with evidence of a proinflammatory response, as indicated by increased proinflammatory innate immune gene expression (LBP, MYD88, IL8, C3). On d 9, NEC incidences became similarly reduced among groups (15-21%), but the CC, FC, and CF pigs showed improved intestinal structure and function, relative to FF pigs (P<0.05). Gene expressions related to tissue hypoxia (HIF1A) and apoptosis (CASP3) were similar on d 5, while HIF1A expression was increased in FC and FF, relative to CC and CF pigs and CASP3 expression was increased in CF and FF vs. CC and FC pigs on d 9 (P<0.05).

Natural milk diets, like COL and MM, may protect the immature intestine from NEC by dampening proinflammatory responses to FOR feeding. Diet-dependent NEC sensitivity is highest within the first week after preterm birth and the diet-dependency may decrease with age. Feeding COL could improve intestinal structure and function for a later FOR-induced insult and repair some damages caused by initial FOR feeding.

COI: Bovine colostrum was donated by Biofiber Damino, Gesten Denmark that had no influence on study design or results interpretation. The University of Copenhagen has filed a patent on the application regarding the use of BC bovine colostrum for pediatric patients with Per Sangild as the sole inventor. He has declined any share of potential revenue arising from commercial exploitation of a such a patent. All other authors have no conflicts of interest.
ID: 291
TITLE: INSULIN-LIKE GROWTH FACTOR 1 IN BRONCHOPULMONARY DYSPLASIA, LATE-ONSET SEPSIS AND NECROTIZING ENTEROCOLITIS
AUTHORS: Yumani DFJ, Walschot FH, Lafeber HN, van Weissenbruch MM
AFFILIATIONS: Emma’s Children Hospital, Amsterdam UMC, Location VU medical center, Vrije Universiteit Amsterdam, Neonatology

CONTENT:

Insulin-like growth factor 1 (IGF-1) is known to influence the development of the premature gut and lungs. In addition, IGF-1 has an anti-oxidative and protective effect on inflammation. The study explores the relation between IGF-1 and the occurrence of bronchopulmonary dysplasia (BPD), late-onset sepsis (LOS) and necrotizing enterocolitis (NEC) in preterm infants.

88 preterm infants born between 24 to 32 weeks of gestation were enrolled in the NUTRIE Study (nutrition in relation to the endocrine regulation of preterm growth). Serum IGF-1 measured at birth and at 2, 4 and 6 weeks postnatal age was compared in infants with and without BPD, LOS and NEC. Mixed models were used to explore the interaction between IGF-1 and the occurrence of LOS and BPD. The models were adjusted for gestational age. Due to the limited patients with NEC (n=8) this could not statistically be explored.

During the first weeks of life preterm infants with BPD (n=30) showed lower IGF-1-levels compared to those without BPD (-1.47; CI -2.18 to -0.75; p < 0.001). Furthermore, IGF-1 levels were significantly lower around the onset of LOS (n=31) (-1.20 nmol/l; CI -2.34 to -0.06; p 0.039). After correction for gestational age these findings only remained significant for BPD.

IGF-1 has a pertinent role in the occurrence of comorbidity in preterm infants as low postnatal levels of IGF-1 are associated with the occurrence of BPD and LOS. A larger study population is needed to explore the interaction between IGF-1 and the development of NEC.

COI: None declared
ID: 370
TITLE: GASTRIC RESIDUALS TO PREDICT NECROTIZING ENTERCOLITIS IN PRETERM PIGLETS
AUTHORS: Susanne Soendergaard Kappel 1,2; Per Torp Sangild 1,2; Thomas Thymann 1; Lise Aunsholt 1,2
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CONTENT:

Background: Necrotizing enterocolitis (NEC) is a serious intestinal inflammatory disease associated with enteral feeding in preterm infants. Volume and color of gastric residual (GR) before each bolus feeding are parameters often used to evaluate feeding intolerance, and used as early predictors of NEC. However, the predictive value of GR evaluations is questionable and may lead to unnecessary withhold of feeding, growth restriction and prolonged use of parenteral nutrition. Using NEC-sensitive preterm piglets as a model of preterm infants, we hypothesized that GR mass, acidity and bile acid levels, and plasma gut hormone levels, may predict early onset of NEC.

Methods: In total, 319 piglets were delivered by caesarean section at 90% gestation (from 20 sows) and fed different cow’s milk-based formulas for 5 days before NEC evaluations and GR collection 60 min after a final bolus feeding. The stomach, small intestine (Si) and colon (Co) were evaluated for NEC lesions by macroscopic scoring (1-2: No-NEC, 3-4: mild lesions, 5-6: severe lesions). GR mass, acidity, gastrin and bile acid levels were measured, and plasma glucagon-like peptide 2 (GLP-2), gastric inhibitory polypeptide (GIP) and gastrin.

Results: Across the piglets, 49% were diagnosed with mild or severe NEC lesions in the Si and/or Co. These piglets (ALL-NEC) had a higher GR mass per body weight than No-NEC piglets (p<0.001). The difference was highest for piglets that had Si lesions (relative to only Co lesions). Presence of NEC lesions was associated with lower gastric bile acid concentrations (p<0.05). The positive and negative predictive values for these markers were 55-65%. No differences were observed for acidity, gastrin, GLP-2 or GIP levels.

Conclusion: In preterm piglets, mild to severe NEC lesions was associated with higher mass of GR but not with the other measured biomarkers of gut function. The predictive value of GR mass is low. It is important that unspecific clinical signs of feeding intolerance and NEC, as judged from GR mass and chemical composition, do not lead to unnecessary reduction in enteral feeding volume for preterm infants.

IMAGES:

Figure 1. Gastric residual (GR, weight of stomach content relative to body weight). a) No-NEC compared to NEC in each of the gastrointestinal regions. b) Gastric bile acid in No-NEC vs. piglets with NEC lesions in the small intestine (Si-NEC, with/without colon lesions) or only in the colon region (Co-NEC). Values are means ± SEMs. * p< 0.05, ** p< 0.01.

COI: None declared
ID: 379

TITLE: PRENATAL ENDOTOXIN EFFECTS ON GUT IMMUNITY AND MICROBIOTA IN PRETERM PIGS

AUTHORS: Xiaoyu Pan 1; Du Zhang 2; Duc Ninh Nguyen 1; Fei Gao 1, 2; Per T. Sangild 1

AFFILIATIONS: 1 Comparative Pediatrics and Nutrition, Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
2 Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen, China

CONTENT:

Gut microbiota exposure plays an important role for intestinal immunity development in preterm neonates. Disruption of the gut homeostasis by inappropriate microbial exposure may increase susceptibility to gut diseases, e.g. necrotizing enterocolitis (NEC). Chorioamnionitis, associated with intra-amniotic infections, may expose the immature intestine to bacterial toxins already in utero via fetal swallowing. Using preterm pigs as model for preterm infants, we hypothesized that prenatal exposure to gram-negative endotoxin influences postnatal gut microbiota and immunity development in preterm neonates.

Pig fetuses were given intra-amniotic lipopolysaccharide (LPS, 1 mg per fetus, n=37) 3 d before preterm delivery by cesarean section, and were compared with litter-mate controls (CON, n=32) at birth and after 5 d of formula feeding. Amniotic fluid was collected for analysis of leukocyte counts and cytokines. The distal small intestine was analyzed for endotoxin level, morphology and immune cell counts. Intestinal gene expression and colonic microbiota were analyzed by transcriptomics and metagenomics, respectively.

At birth, LPS-exposed pigs showed higher intestinal endotoxin, neutrophil/macrophage density and lower villi. About 1.0% of intestinal genes were affected and DMBT1 (deleted in malignant brain tumor 1, a regulator of mucosal defense) was a hub gene in the co-expression network. Gene expressions related to innate immune response (TLR2, LBP, CD14, C3, SFTPD), leukocyte transendothelial migration (NCF1, NCF2, NCF4, ITGB2) and antigen processing (MHC II, CD4) were changed and correlated with intestinal neutrophil/macrophage density and amniotic fluid cytokine levels. On day 5, LPS and CON pigs showed similar NEC lesions, endotoxin levels, morphology, immune cell counts and gene expressions, but differences in low-abundant Lactobacillus amylovorus (~0.05% of total bacteria in colon).

LPS exposure affected the expression of intestinal genes in preterm pigs at birth, especially genes related to immune cell infiltration. Five days later, following enteral feeding and bacterial colonization, intra-amniotic LPS had limited effects on intestinal structure and function. A short period of intra-amniotic inflammation prior to preterm birth is unlikely to cause longer-lasting pro-inflammatory responses in the gut of preterm infants.

COI: None declared
ID: 486

TITLE: STEM CELLS FOR THE PREVENTION OF EXPERIMENTAL NECROTIZING ENTEROCOLITIS: A SYSTEMATIC REVIEW AND
META-ANALYSIS OF PRECLINICAL STUDIES

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4 Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE), Department of Health Evidence, Radboud
University Medical Center, Nijmegen, The Netherlands

CONTENT:

Necrotizing enterocolitis (NEC) is the leading cause of morbidity and mortality from gastrointestinal disease in very and
extremely preterm infants. At present, there are no specific therapies for NEC. Stem cell therapy has shown promising
protective effects in animal models of intestinal injury, including NEC. Animal models are invaluable tools for enriching our
understanding of the pathogenesis and treatment of human diseases but systematic reviews and, where appropriate, meta-
analyses are required to summarize the pre-clinical evidence on a given subject. No systematic review has yet evaluated
the preclinical evidence of stem cell therapy for NEC prevention/treatment.

PubMed/Medline and EMBASE databases were searched for relevant articles published through October 2018, and
electronic alerts were set up to inform us of studies published during the elaboration of the review. Studies were included if
they used an animal model of NEC with stem cells or their products as the intervention, according to a previously registered
protocol at PROSPERO (ID: 110084). Risk of bias was critically appraised using the SYRCLE Risk of Bias Tool for Animal
Studies. To increase reliability, two independent reviewers included studies, collected outcome data and appraised risk of
bias. A random-effects model was used to calculate odds ratios (OR) or standardized mean differences, as appropriate. We
used the PRISMA guidelines for reporting.

We screened 953 non-duplicate studies, of which 9 (8 rat models and 1 mouse model) met the inclusion criteria. Risk of
bias was evaluated as unclear on most items for all studies included. Meta-analysis found that stem cells improved 4-day
survival (OR 2.89 95% CI 2.07-4.04) and 7-day survival (OR 3.96 95% CI 2.39-6.55), reduced the incidence of all NEC (OR
0.26 95% CI 0.19-0.35), grade 2 NEC (OR 0.44 95% CI 0.27-0.71), and grade 3-4 NEC (OR 0.28 95% CI 0.19-0.42). Meta-
analysis also found that stem cells reduced other indicators of intestinal injury. Subgroup analyses for stem-cell type
(mesenchymal, neural or amniotic fluid stem cells) could not find significant differences between subgroups.

Results from this systematic review and meta-analysis of pre-clinical studies suggest that stem cell therapy may be a
promising treatment option for infants with NEC. However, unclear risk of bias and incomplete reporting underline that
poor reporting standards are common and hamper the pre-clinical evidence for stem cell therapy for NEC. Better reporting
and trials in other species are required before implementation of human trials.

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Figure. Random effects meta-analysis of stem cell therapy and incidence of necrotizing enterocolitis.
AFNSC: Amniotic Fluid Neural Stem Cells; AFNSC: Amniotic Fluid Mesenchymal Stem Cells; BMMSC: Bone Marrow Mesenchymal Stem Cells; CI: confidence interval; ENSC: Embryonic Neural Stem Cell; exp: experiment; hBMMSC: Human Bone Marrow-derived Mesenchymal Stem Cells; IP: Intraperitoneal; IV: Intravenous; NSC: Neural Stem Cells.

COI: None declared
ID: 598

TITLE: CAN SPLANCHNIC NEAR INFRA-RED SPECTROSCOPY BE USED AS A VALID TOOL IN NEONATOLOGY?

AUTHORS: Emilie Seager (first author) 1, Catherine Longley 1, Narendra Aladangady 2, Jayanta Banerjee 3

AFFILIATIONS: 1 - Imperial College Healthcare NHS Trust
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3 - Imperial College Healthcare NHS Trust and Imperial College London

CONTENT:

Near infrared spectroscopy (NIRS) measures the amount of oxygenated and deoxygenated haemoglobin, using venous, arterial and capillary haemoglobin in tissues, thereby measuring regional tissue oxygen saturation. NIRS was first demonstrated to be valuable in measuring brain oxygenation in 1977; it is routinely used to measure brain oxygenation in cardiac surgery and ITU. However, its usefulness in measuring gut perfusion remains to be proven. We undertook a systematic review to determine whether NIRS can be deemed a valid tool to measure regional oxygenation of gut tissue in neonates.

Pub Med and Embase databases were searched using the following terms: ‘preterm infants’, ‘NIRS’, ‘neonate’ and ‘gut oxygenation’. Where the title suggested relevance to the review the full article was reviewed to determine whether it was to be included. All clinical trials, observational studies and experimental animal studies that examined the validity of NIRS were included. Abstracts not in English and not relating to gut oxygenation were not included. This search was performed by two independent reviewers and the results then reviewed by two further independent authors.

A few studies have assessed the validity of NIRS measuring gut oxygenation (Table 1). The relationship between ileal blood flow, NIRS and liver tissue oxygenation has been well described; however, there is limited data regarding splanchnic NIRS and gut perfusion. Splanchnic NIRS has been compared to gastric pH by Kaufman et al who found a strong correlation between the two (r=0.79, p<0.0001). Fortune et al used abdominal NIRS to determine splanchnic perfusion in comparison to cerebral perfusion and demonstrated that the cerebro-splanchnic oxygenation ratio (CSOR) was highly predictive of intra-abdominal pathology such as necrotizing enterocolitis (NEC).

NIRS has been studied in detail in the neonatal population and can be used to monitor gut perfusion. Gut NIRS should be used as a tool in future randomised trials to underpin its use in clinical practice. Further research into normal values in different neonatal populations and a consensus on interpretation of results may facilitate adopting NIRS as part of routine, non-invasive bed side monitor in neonatal units soon.

IMAGES:
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Table 1

COI: None declared
ID: 848  
TITLE: SURGICAL INTERVENTION IN NEONATES WITH NECROTISING ENTEROCOLITIS: A RISK STRATIFICATION SCORE  
AUTHORS: Shazia P Sharif, Rafdzah A. Zaki, Michael F. Hird, Simon R. Phelps  
AFFILIATIONS: 1, 4: Department of Paediatric Surgery, The Royal London Children's Hospital, London UK  
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CONTENT:  
Necrotising enterocolitis (NEC) is the commonest surgical condition affecting premature infants. It still carries a significant morbidity and mortality despite advances in neonatal intensive care. Early risk stratification of infants who will require surgery for NEC is important as this enables more informed parent counselling, a higher index of clinical suspicion of the need for surgical intervention and/or prompt transfer to a surgical centre. Stratification would also reduce unnecessary neonatal transfers and improve neonatal cot utilisation in surgical centres.

Part 1 of the retrospective study was undertaken of a prospectively-collected electronic neonatal database (Badgernet) including all neonates with NEC modified Bell Stage 2 between 2009 – 2015 in one centre. Data on patient demographics, clinical parameters, specific laboratory findings through the episode of illness (serum white cell count, platelet count, C-RP and albumin), abdominal radiographs, surgical status and death were recorded. Multivariate analysis was performed to identify significant factors and a scoring system was developed. Part 2 of the study collected the same data between 2016 - 2018. Data from the second part was used to validate the scoring system developed in part 1.

Part 1 of the study included 133 infants. Analysis of the data identified factors significantly associated with surgery. Factors included in the final model were: male gender, extremely low birth weight (<1000g), serum albumin < 20g/L on day 2, platelet count < 100 x 10^9/L on day 2, white cell count < 5 x 10^9/L on day 2 and C-RP > 20mg/L on day 2. A final risk scoring system was developed with maximum total score of 10. A score of 6 or more indicates the probability of need for surgery is > 70% and has a predictive value with AUC=71.8%.

Part 2 of the study included 64 infants. The identified score variables for these infants were entered into the scoring system to predict the need for surgery. Predicted outcomes were compared with the actual outcomes for this set of infants. A risk score of 6 or more out of 10 in the proposed scoring system has a predictive value of 71.8% for need for surgery with a positive predictive value (PPV) of 83.3% and negative predictive value (NPV) of 80.5% (p =0.004).

The scoring system is a tool for stratifying need for surgery for an infant with NEC modified Bell stage 2. It should be used as an adjunct to clinical assessment and judgement and is not designed as a clinical substitute. To validate this further, we plan to conduct a prospective observational study with larger sample size in conjunction with the London Neonatal Transport Service.

COI: None declared.
ID: 879  
TITLE: NEGLECTED AND DESPERATELY NEEDED: NUTRITIONAL SUPPORT FOR PRETERM SURVIVAL IN LOW-INCOME COUNTRIES  
AUTHORS: Nicole Rouvinez Bouali 1; Marcelline D’Almeida 2; Nicole Tchiakpe 3; Noe Akonde 3; Lehila Bagnan 2; Maroufou Jules Aalo 3; Blaise Ayivi 2.  
AFFILIATIONS: 1 Department of Pediatrics, University of Ottawa, Ontario, Canada  
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3 Division of Neonatology, Department of Pediatrics, Centre Hospitalier Universitaire de la Mère et de l'Enfant Lagune (CHUMEL) and University of Abomey-Calavi, Cotonou, Benin  

CONTENT:  
Neonatal mortality represents 45% of under-5-year-old deaths worldwide and malnutrition contributes to half of those deaths. In Benin, like in other Sub-Saharan African countries, prematurity (35%), neonatal infections (26%) and birth-related events (28%) explain 90% of all neonatal deaths. Exclusive breastfeeding at 6 months is only 40% and the availability of breastmilk for hospitalized newborns is limited. Preterm infants are particularly at risk of complications due to malnutrition. Kangaroo Mother Care is recommended by WHO. We report on interventions done during the “Breath of Life-Benin” (BOL) programme to improve the nutritional status and survival of hospitalized preterm infants.  

BOL-Benin is a multifaceted program targeting the main causes of neonatal mortality. It was developed and implemented by our Global Development Alliance and co-funded by USAID Benin (2015-2017). Interventions to reduce mortality due to prematurity included, amongst others, emphasis on early nutrition with mother’s own breast milk and continuous Kangaroo Mother Care (KMC). We report the survival (number and percent), length of stay (days) and weight gain/loss (grams and percentage below/above birth weight) observed in preterm infants admitted to the neonatal unit and KMC unit, according to birth weight (BW) categories (1000-1249 grams (g), 1250-1499 g, 1500-1749 g and 1750-1999 g), in the 2 largest neonatal units of Benin, before and after BOL.  

Approximately five thousand newborns are admitted each year between the 2 neonatal units, 60% of them being preterm infants, with approximately 2/3 inborn and 1/3 outborn. Before BOL, skin-to-skin care was limited to breastfeeding attempts for more mature infants. Survival was dismal below a BW of 1250 g and uncommon below 1500 g and malnutrition was present in virtually all preterm infants surviving beyond a week of age. Following BOL interventions, preterm survival increased significantly for infants with BW below 1500 grams. Despite earlier feeds initiation, increased breast milk ratio and protein supplementation, preterm infants cared for in the neonatal unit exhibited poor weight gain. Preterm infants cared for in the KMC unit 24h/day demonstrated constant weight gain, significantly improved survival (beyond 95% for infants 1000-1999 grams) and earlier discharge home.  

BOL interventions improved survival of preterm infants but malnutrition remains a concern in the neonatal unit. KMC increased survival and weight gain of low BW infants. Future efforts to improve preterm infants’ outcome in low-income countries should emphasize on KMC and consider involvement of families at bedside to palliate understaffing in the neonatal unit. Parenteral nutrition and milk fortification may be needed in very low BW infants.  

COI: None declared
ID: 953  
TITLE: THE IMPACT OF FEEDING REGIMES ON ENDOGENOUS LEVELS OF IGF-1 IN THE PRETERM RABBIT PUP  
AUTHORS: William Hellström 1; Kristbjorg Sveinsdottir 2; Suvi Vallius 2; Susanne Grönlund 2; Helena Karlsson 2; Matteo Bruschettini 2; David Ley 2  
AFFILIATIONS: 1 Department of Pediatrics, Institute of Clinical Sciences, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden  
2 Department of Pediatrics, Institute of Clinical Sciences Lund, Lund University and Skane University Hospital, Lund, Sweden  

CONTENT:  
Serum levels of insulin growth factor 1 (IGF-1) are low in the preterm infant compared to term. IGF-1 is linked to metabolic and neuro developing pathways and brain growth, and the role of IGF-1 on organ development has been widely investigated. Due to lagomorphs comparably late cerebral development and early lung development in fetal life, the preterm rabbit model serves as an excellent platform for research addressing preterm brain development and injury. The aim of this investigation was to evaluate serum levels of IGF-1 in two different feeding regimes of potentially central importance during early postnatal development.

In total 70 preterm rabbit pups were included, 38 in the wet nurse (W) regimen and 32 in the formula fed (F) regimen. 46 term pups were included. Preterm pups were delivered by C-section at E29 (three days prior to term age). Blood samples were collected at E29, P0 (=3 days after C-section), P2, P5, P9 and P10 and serum concentration of IGF-1 was determined with ELISA. In W, pups were given 0.5 ml of bovine colostrum once and then placed with a foster doe in a litter size ranging from 7-8 pups in total. In F, pups were hand-fed with kitten-milk replacement formula, using a 3.5 Fr feeding tube; the first feeding at 2 h of age at a volume of 1 mL and then every 12 h achieving an incremental increase from 70 to 250 ml/kg/d. The term pups were housed with and received milk from their mothers.

Serum levels of IGF-1 at day E29, P0, P2, P5, P9 and P10 are illustrated in Figure 1. Serum levels were significantly lower in the formula fed group compared to preterm pups in the wet nurse nutritional regime at P0 (p=0.001) and lower compared to term pups at P0, P2, P9 respectively (p=0.021, p=0.001, p=0.004). Preterm pups fed by wet nurse exhibited levels of IGF-1 corresponding to those of healthy term pups when adjusting for gestational age. Serum levels of IGF-1 correlated with weight development irrespective of nutritional regime; formula fed preterm pups (p=0.006, rSpearman=0.487), pups fed by wet nurse (p=<0.001, rSpearman=0.950) and term pups (p=<0.001, rSpearman=0.843).

Serum levels of IGF-1 were clearly affected by feeding regime in preterm rabbits showing that clinically relevant modifications of feeding and nutrition have a strong potential to modify alterations in the central IGF-1 pathway normally associated with preterm birth. Continued study will address several potentially influencing components of maternal care beyond those of nutritional content.

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Serum levels of IGF1 in the wet nurse nutritional regimen were low and comparable with endogenous levels of IGF-1 in term pups adjusting for age. Rabbit pups in the formula fed nutritional regimen had significantly lower levels of serum IGF-1. Error bars represent 95% CI.  
IGF-1 = Insulin like growth factor 1, CI = confidence interval  

COI: None declared
ID: LATE BREAKER
TITLE: COMMERCIALY PROCESSED DONOR HUMAN MILK-DERIVED PRODUCTS RETAIN A FULL-SPECTRUM OF FUNCTIONALLY ACTIVE OLIGOSACCHARIDES

AUTHORS:

AFFILIATIONS:

CONTENT:

Background: Human milk (HM) contains many bioactive substances, including a family highly abundant and structurally unique glycans, called human milk oligosaccharides (HMOs). HMOs constitute the third most abundant component in HM, following lactose and fat, and are more abundant than protein. The best-characterized function of HMOs is their prebiotic activity, which supports the colonization of the gut by mutualist bacteria in the period after birth. Bifidobacterium spp. and other, commensal bacteria may reduce diseases like NEC and sepsis by supporting gut colonization by commensal bacteria over pathobionts. We sought to explore the impact of commercial vat pasteurization and ultrafiltration on the concentration, spectrum, and activity of HMO present in donor HM and a human milk-derived HM protein fortifier.

Methods: HPAEC-PAD with 11 oligosaccharide standards were used to annotate the spectrum and extrapolate the concentration of HMO extracted from native and pasteurized donor HM and a HM-derived, HM protein fortifier. I-screen assays (TNO, Zeist) were performed in triplicate using fecal matter prepared from infant, adult or aged donors. Serial dilution of a HM-extract were added from ~3.0 mg/ml to 0.03 mg HMO per well and incubated at 37C for 24 hours under anaerobic conditions. Bacterial DNA was isolated, 16S V4 rRNA amplicons prepared and sequenced using Illumina MiSeq with downstream analysis using a standardized pipeline. Assembled reads and taxonomic classification was performed using Ribosomal Database Project (University of Illinois, Urbana) with composition presented as OTU. The supernatant of individual wells was collected, and small chain fatty acids were enumerated using gas chromatography and mass spectroscopy.

Results: We identified a similar spectrum of neutral and acidic HMO in a pasteurized donor HM, and human milk-derived HM protein fortifier similar to native donor HM tested before processing. There were no statistically significant differences in the concentration of total, neutral, or acidic HMO across the product pipeline. Statistically significant increases in OTU specific for Bifidobacterium spp. moreover, commensal Clostridial spp. were found in all fecal pools although infant fecal pools resulted in a more robust response in Bifidobacterium spp. than did fecal pools from adult or aged adults. When the composition of small chain fatty acids was analyzed, acetate and butyrate were identified in adult and aged fecal samples whereas only acetate was identified in infant fecal pools.

Conclusions: Commercial processing of HM, including vat pasteurization, freeze-thaw, and ultrafiltration preserves the content, spectrum, and activity of naturally occurring HMOs with ex vivo fidelity of prebiotic activity and SCFA production. Our data support that supplementation and fortification of mother’s milk or donor HM complement the bioactivity of a smaller assortment of HMOs. Ongoing clinical studies to determine the impact on exclusive human milk feeding con the microbiome of preterm newborns are in progress.
ID: LATE BREAKER

TITLE: EARLY PARENTERAL NUTRITION USE AND OUTCOMES IN PRETERM NEONATES

AUTHORS:

AFFILIATIONS:

CONTENT:

Background
Preterm babies are among the highest users of parenteral nutrition (PN). The evidence underpinning PN use is low quality and neonatal trials, powered for clinically meaningful end-points, have not evaluated impact on key outcomes. There is therefore wide variation in commencement, duration, and composition. Recent studies in critically ill adults and children show that harms, particularly increased rates of nosocomial infection, outweigh the benefits of early PN administration. We aimed to evaluate whether in a population of very preterm neonates, PN use in the first seven postnatal days, compared with no PN use, in the rate of survival (and other important morbidities) is higher.

Methods
We prospectively registered this study (NCT03767634) and published the protocol. We used data from the UK National Neonatal Research Database to compare outcomes in neonates born between 30+0 and 32+6 weeks gestation, from January 2012 to December 2017, and admitted to a neonatal unit in England and Wales. The intervention was any administration of PN in the first seven postnatal days. The primary outcome was survival to discharge home. Secondary outcomes comprised the neonatal core outcome set; outcomes considered vital by over 400 former patients, parents, clinicians and researchers.

To minimise bias and confounding, we used propensity matching including 35 maternal, infant and organisational factors in the model. We calculated absolute risk differences for the outcomes.

Results
Over the study period, there were 37,302 babies born between 30+0 and 32+6 gestation in England and Wales of which 36,644 were admitted to a neonatal unit. After propensity matching 8,207 pairs were formed. The pairs were well matched on principal background variables (Table 1), with a standardised mean difference of 0.013. Survival was higher in the group that received PN (survival: ‘PN’ 98.8%; ‘No PN’ 97.3%; absolute risk reduction 1.54, 95% confidence interval 1.30, 1.76). However, the group that received PN also showed increased morbidity (bronchopulmonary dysplasia: ‘PN’ 9.9%; ‘No PN’ 4.4%; absolute risk increase 5.5%; 95% confidence interval 4.9, 6.1; late-onset sepsis: ‘PN’ 2.5%; ‘No PN’ 1.1%; absolute risk increase 1.4%; 95% confidence interval 1.0, 1.6).

Conclusion
We found that babies that received PN in the first postnatal week had improved survival, but an increase in major morbidities. The extensive background variables used in matching reduced the risk of confounding, but as this was an observational study, we cannot exclude the risk of residual and unmeasured confounding. We suggest that our data add to growing justification for a well-powered randomised clinical trial evaluating long-term outcomes.

Table 1  Principal background variables used in the propensity score matching

<table>
<thead>
<tr>
<th>Entire cohort</th>
<th>Matched cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>No PN group</td>
<td>(N=19,168)</td>
</tr>
<tr>
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<td>(N=16,076)</td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td>(N=8,207)</td>
</tr>
<tr>
<td></td>
<td>PN group</td>
</tr>
<tr>
<td></td>
<td>(N=8,207)</td>
</tr>
</tbody>
</table>

Missing data Missing data Missing data Missing data

Gestational age (weeks), mean (SD) 32.0

(4.4) 0 31.3
(0.9) 0 31.1
(0.8) 0 31.1
(0.8) 0 
Birthweight (kg), mean (SD) 1.74 
(0.28) 6 1.47 
(0.32) 5 1.67 
(0.27) 0 1.58 
(0.29) 0 
Proportion small for gestational age, n (%) 839 
(4.4) 6 3762 
(23.4) 5 1354 
(16.5) 0 1354 
(16.5) 0 
Infant sex, n (%) 
Male 10326 
(53.9) 0 8754 
(54.5) 0 4481 
(54.6) 0 4465 
(54.4) 0 
Female 8842 
(46.1) 0 7322 
(45.5) 0 3726 
(45.4) 0 3742 
(45.6) 0 
Antenatal steroids, n (%) 
Yes, complete course 3175 
(17.4) 920 2377 
(15.4) 643 1249 
(15.9) 468 1261 
(16.0) 413 
Yes, incomplete course 12354 
(67.7) 920 11081 
(71.8) 643 5553 
(70.7) 468 5586 
(70.9) 413 
No 2719 
(14.9) 920 1960 
(12.7) 643 1052 
(13.4) 468 1032 
(13.1) 413 
Umbilical arterial pH, mean (SD) 7.3 
(0.1) 11346 7.3 
(0.1) 9431 7.3 
(0.1) 6018 7.3 
(0.1) 5968 
Apgar score at 5 minutes, 
mean (SD) 8.8 
(1.3) 1616 8.4 
(1.6) 1448 8.7 
(1.4) 868 8.4
Intubation during resuscitation, n (%) 1668
(1.6) 934
(8.7) 0 3247
(20.2) 0 1518
(18.5) 0 1494
(18.2) 0

Admission temperature, mean (SD) 36.9
(3.3) 107 36.9
(8.2) 85 36.8
(1.9) 54 36.9
(3.0) 61

Admission heart rate, mean (SD) 157
(19) 1199 156
(22) 1145 157
(21) 627 157
(25) 719

Admission blood sugar, mean (SD) 3.1
(1.9) 3835 2.8
(4.4) 2879 3.0
(3.4) 1964 2.9
(2.3) 1806

Ventilated on first day, n (%) 699
(7.5) 9852 1500
(17.6) 7556 669
(15.9) 3997 678
(16.0) 3972

Treated for sepsis on first day, n (%) 911
(21.8) 14989 973
(24.6) 12121 457
(23.6) 6270 473
(24.0) 6237

Level of neonatal unit, n(%)
Level 1 (LNU) 2588
(13.5) 0 1190
(7.4) 0 673
(8.2) 0 624
(7.6) 0
Level 2 (SCBU) 8319
(43.4) 0 7556
(47.0) 0 4005
(48.8) 0 3948
(48.1) 0
Level 3 (NICU) 8031
(41.9) 0 7170
(44.6) 0 3431
(41.8) 0 3537
(43.1) 0

Transferred on first day, n (%) 1093
(5.7) 0 1334
(8.3) 0.624
(7.6) 0.648
(7.9) 0

LNU = Local neonatal unit; SCBU = Special care baby unit; NICU = Neonatal intensive care unit