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Enteral but not parenteral antibiotics enhance gut function and prevent necrotizing enterocolitis in formula-fed newborn preterm pigs

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Birck MM, Nguyen DN, Cilieborg MS, Kamal SS, Nielsen DS, Damborg P, Olsen JE, Lauridsen C, Sangild PT, Thymann T. Enteral but not parenteral antibiotics enhance gut function and prevent necrotizing enterocolitis in formula-fed newborn preterm pigs. *Am J Physiol Gastrointest Liver Physiol* 310: G323–G333, 2016. First published December 17, 2015; doi:10.1152/ajpgi.00392.2015.—Preterm infants are susceptible to infection and necrotizing enterocolitis (NEC) and are often treated with antibiotics. Simultaneous administration of enteral and parenteral antibiotics during the first days after preterm birth prevents formula-induced NEC lesions in pigs, but it is unknown which administration route is most effective. We hypothesized that only enteral antibiotics suppress gut bacterial colonization and NEC progression in formula-fed preterm pigs. Caesarean-delivered preterm pigs (90–92% of gestation) were fed increasing amounts of infant formula from birth to *day 5* and given saline (CON) or antibiotics (ampicillin, gentamicin, and metronidazole) via the enteral (ENT) or parenteral (PAR) route ($n = 16$–17). NEC lesions, intestinal morphology, function, microbiology, and inflammatory mediators were evaluated. NEC lesions were completely prevented in ENT pigs, whereas there were high incidences of mild NEC lesions (59–63%) in CON and PAR pigs ($P < 0.001$). ENT pigs had elevated intestinal weight, villus height/crypt depth ratio, and goblet cell density and reduced gut permeability, mucosal adherence of bacteria, IL-8 levels, colonic lactic acid levels, and density of Gram-positive bacteria, relative to CON pigs ($P < 0.05$). Values in PAR pigs were intermediate with few affected parameters (reduced lactic acid levels and density and adherence of Gram-positive bacteria, relative to CON pigs, $P < 0.05$). There was no evidence of increased antimicrobial resistance following the treatments. We conclude that enteral, but not parenteral, administration of antibiotics reduces gut bacterial colonization, inflammation, and NEC lesions in newborn, formula-fed preterm pigs. Delayed colonization may support intestinal structure, function, and immunity in the immediate postnatal period of formula-fed preterm neonates.

antibiotics; necrotizing enterocolitis; enteral antibiotics; preterm pig

EVERY YEAR, APPROXIMATELY 15 million infants are born preterm worldwide (i.e., before the completion of 37 wk of gestation), comprising 11% of all pregnancies (42). These infants have an immature gastrointestinal (GI) tract and immune system and are therefore susceptible to infection and intestinal diseases, such as necrotizing enterocolitis (NEC). NEC is the most common GI complication affecting 7% of very low birth weight infants (500-1,500 g) with a mortality rate of 20–30% (31). The etiology and pathogenesis of NEC are still incompletely understood, but prematurity, aggressive enteral nutrition, and abnormal gut bacterial colonization are important predisposing factors (37).

Breast milk is the most NEC-protective diet for preterm infants (43), but lactation is often delayed and mother's milk is frequently inadequate for enteral nutrition after preterm delivery within the first week. Conversely, alternatives to breast milk, such as infant formula, are associated with increased NEC incidence (6–10 times, 28). Therefore, it is critical to identify medical and nutritional strategies to protect formula-fed preterm infants against NEC development. Modulations of the gut microbiota with pre- and probiotics may decrease NEC risk, but important questions related to type and dose of different products and their optimal time of administration remain unclear (10, 18, 23, 46).

Intravenous antibiotics are frequently used in neonatal intensive care units (NICUs) to prevent and treat bacterial infections caused by invasion via indwelling catheters or via bacterial translocation from the immature gut or other body surfaces (27). Likewise, intravenous antibiotics are commonly applied as a treatment following the onset of NEC (31). A systematic review of five studies including 456 preterm infants revealed that enteral antibiotics reduced NEC incidence and NEC-related deaths in low birth weight infants (5). However, these studies have not led to a widespread use of prophylactic enteral antibiotics for preterm infants, in part attributable to uncertainties regarding product, time, doses, and efficacy to promote intestinal health. Other important reservations are the increased risk of NEC after long-term use of antibiotics and the possible development of antibiotic resistance (1, 11, 22). Regardless, it remains unknown how a few days of neonatal antibiotics affect host response, antibiotic resistance, and total need for antibiotics during hospitalization.

The preterm pig is a preclinical model of preterm infants in which the immature gut is highly sensitive to both microbial colonization and formula feeding. Thus preterm pigs fed rapidly advancing volumes of enteral formula within the first week after birth by caesarean section at 90–92% gestation have a high incidence of spontaneous NEC-like lesions (38). Even a gradual introduction of formula feeds causes mild NEC le-

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