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Pediatric RESEARCH

COMMENT

Human breast milk exosomes may protect against necrotizing enterocolitis in preterm infants

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As a neonatologist and international board-certified lactation consultant, I read this month's article by He, Liu, and Zhu, "Human breast milk-derived exosomes may help maintain intestinal epithelial barrier integrity," with great interest. The myriad benefits of human breast milk for our preterm infant population includes improved neurocognitive outcomes, lower risks of infection, less severe retinopathy of prematurity, lower rates of bronchopulmonary dysplasia, and decreased lengths of stay in neonatal intensive care units (NICUs). In addition, preterm infants who receive exclusive breast milk diets are at a much lower risk of the development of necrotizing enterocolitis (NEC) than those who are formula fed.

NEC is an inflammatory disorder of the intestinal tract that occurs predominantly in preterm, very-low-birthweight (VLBW, birthweight < 1500 g) infants during the first 4–6 weeks of extrauterine life. Patients with NEC present with abdominal distension, feeding intolerance, increased gastric aspirates, and a classic radiographic finding called pneumatosis intestinalis, which gives the bowels a "bubbly" appearance. Severe cases of NEC can lead to bowel perforations and strictures, which often require surgery, and carry a high risk of mortality. Historically, 30–50% of preterm patients with NEC have died. When I started my neonatal–perinatal fellowship training 15 years ago, 10–12% of my VLBW patients developed NEC and it was the most common cause of death amongst VLBW infants. To this day, it still remains difficult to predict which NICU patients will develop NEC. I have cared for many preemies who are stable and well appearing during our morning rounds, only to die <24 h later from a rapid clinical deterioration from fulminant NEC.

NEC is associated with increased epithelial permeability of intestinal cells, which is associated with diffuse inflammation and damage. Despite decades of research, the true "root cause" of NEC has yet to be delineated. Multiple factors may contribute to the intestinal wall destruction seen in NEC, including infection, decreased gut perfusion in utero, bowel ischemia, growth restriction, an imbalance of intestinal microbiota, and formula feeding.

NEC is much more common in preterm infants who are formula fed than those who receive breast milk. VLBW patients who are given infant formula develop NEC twice as often as VLBWs who are on exclusive human breast milk diets. Breast milk is so essential for feeding our smallest and most fragile NICU patients that many of us refer to it as "liquid gold" when we counsel our NICU patients' mothers about the importance of breast pumping and providing milk for their babies' overall health and to prevent NEC.

There has fortunately been a decrease in severe cases of NEC nationwide as the use of donor breast milk has replaced preterm formula as the "standard of care" for supplementing maternal breast milk feeds. In recent years, the rate of NEC amongst VLBW infants has been 2–7%. Although the incidence of NEC is lower than it used to be, we still lose far too many preterm infants to this tragic disease every year.

While we have ample data supporting that VLBW infants benefit from 100% human milk-based diets, the precise reason why breast milk is protective against NEC has remained a mystery. Human breast milk contains numerous components that enhance infants' immune and digestive function, including lactoferrin, human milk oligosaccharides, and beneficial bacteria, such as *Lactobacillus* sp. and *Bifidobacterium* sp. Although many of these "ingredients" in breast milk have been shown to play a role in improving babies' intestinal integrity and immune function, none of them have been deemed the "magic bullet" for preventing NEC.

In this issue of *Pediatric Research*, we enter into the next frontier of NEC research with the authors' discovery that exosomes found in human breast milk are associated with enhanced intestinal integrity.

So, what exactly are exosomes? Exosomes are vesicles that are released from cells when the multivesicular body (an intermediate endocytic compartment) fuses with cells' plasma membranes. Unlike other cell components, such as the nucleus and Golgi body, there is not a reliable marker for exosomes because the multivesicular body is constantly in a state of flux. Due to their small size (100–200 nm), exosomes can only be seen under electron microscopy and are isolated from body fluids by differential centrifugation.

Despite challenges in visualizing and isolating exosomes, there has been an increased interest in research related to exosomes in recent decades. Based on previous research, exosomes are known to have the following purposes:

1. Exosomes are a major means of intercellular communication.
2. Exosomes play a role in the spread of lipids, proteins, messenger RNA, microRNA, and DNA throughout the body.
3. Exosomes can be vectors for drugs as a result of being composed of cell membranes.

Previous research has demonstrated that human breast milk is exosome-rich and that the exosomes in milk have protective effects on intestinal cells. The authors of this month's paper were the first to compare the levels of exosomes in human breast milk

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from mothers of preterm infants versus mothers of full-term infants. The fact that they did not find a difference in levels of exosomes in preterm versus term breast milk is important, as many VLBW infants do end up receiving donor breast milk from mothers of full-term infants. It is reassuring to know that full-term donor breast milk is chock-full of beneficial exosomes.

The authors also investigated the effect of exosomes on inflammation of intestinal cells, both in vitro and in vivo. Lipopolysaccharide (LPS) promotes intestinal inflammation by attacking epithelial tight-junction proteins (ZO-1, claudin 1, and occludin) and is implicated in NEC. In this study, LPS-stimulated human intestinal cells, simulating the inflamed intestinal cells in neonatal NEC, which were pre-treated with exosome-containing breast milk maintained their tight-junction proteins.

These findings of the protective effects of exosome-containing human milk on intestinal cells were also demonstrated in the authors' experiments on rat pups. Rat pups who received exosome-containing human milk prior to being induced with NEC had significantly less intestinal inflammation and mucosal injury than rat pups who received either no breast milk or "exosome-free" breast milk prior to NEC induction. The presence of exosomes might be the missing piece of the puzzle as to why human milk-based diets play such a critical role in protecting VLBW infants from NEC.

We will need future research to investigate if giving human milk-derived exosomes to preterm babies can actually prevent NEC in the NICU setting. Exosome treatment could ultimately be of great benefit in NICUs where donor milk is not available and/or is not culturally accepted. It is also possible that in the future, all preterm infants, even those who are given their mothers' own milk, might be able to receive a "booster" dose of exosome therapy within the first days of life for extra NEC prevention.

There are many additional future possibilities for research on human milk exosomes. It will be important to figure out whether levels of exosomes differ between maternal breast milk versus donor breast milk, which is either pasteurized or retort processed prior to administration. It would also be fascinating to investigate if human breast milk exosome levels vary based on maternal factors such as genetics, diet, baseline state of health, medications or supplements received, how mothers were fed as infants, mode of infant delivery, etc. In addition, it is possible that human milk-derived exosomes may ultimately be found to play a role in the prevention and treatment of other pediatric inflammatory intestinal disorders associated with impaired tight-junction proteins, including food protein-induced enterocolitis syndrome, milk-soy protein allergies, celiac disease, and Crohn's disease. Although these digestive diseases are not associated with the same mortality risk as NEC, they do have a significant impact on health care utilization and overall quality of life for patients and their families.

REFERENCE

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