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Milk Epidermal Growth Factor and Gut Protection

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Abstract

Maternal milk is a complex fluid with multifunctional roles within the developing gastrointestinal tract. Epidermal growth factor (EGF) and heparin-binding EGF-like growth factor (HB-EGF) are members of the family of EGF-related peptides. Biological actions of these growth factors are mediated via interaction with the EGF-receptor (EGF-R). In the early postnatal period, breast milk is the major source of EGF for the developing intestinal mucosa. HB-EGF is also detected in breast milk, but in concentrations 2 to 3 times lower than EGF. Under normal physiological conditions, the intestinal epithelium undergoes a continuing process of cell proliferation, differentiation and maturation. EGF plays an important role in these processes. In pathophysiologic situations, EGF contributes to epithelial protection from injury and post-injury mucosal repair. Necrotizing enterocolitis (NEC) is a devastating disease affecting prematurely born infants. The pathogenesis of NEC is not known and there is no effective treatment for this disease. In an experimental NEC model, oral administration of a physiological dose of EGF significantly reduces the incidence and severity of NEC. HB-EGF provides similar protection against NEC, but only when pharmacological doses are used. Further studies are necessary before EGF can be introduced as an efficient therapeutic approach of intestinal injury.

Maternal milk and developing gut

Maternal milk is an important and well-balanced source of nutrition for the newborn (1). Milk provides not only major nutrients, vitamins and minerals, but also a plethora of biologically active substances, such as hormones, cytokines, and growth factors (2-6). These biological active factors not only facilitate the development of essential digestive functions, but also regulate the maturation of the intestinal mucosal barrier (7). In addition to their normal physiological functions, many of these factors have the capacity to stimulate the healing and repair processes in injured intestinal epithelium.

The gastrointestinal (GI) tract is a complex organ which undergoes substantial changes during the early stages of development. Profound growth, morphological and functional changes are observed during the late gestation and early postnatal period (8). Birth is possibly the most critical period in gastrointestinal development, when placental supply is replaced by enteral nutrition. Enteral nutrition initiates changes in intestinal mucosal structure and function required for utilization of milk feedings. However, intestinal maturation begins long before birth and amniotic fluid plays an important role in this process (7). In addition to its digestive

function, the GI tract serves as a barrier to the external environment and its epithelium must be renewed rapidly and repeatedly. All these processes are controlled by a number of regulatory agents originating from both endogenous and exogenous sources, including amniotic fluid and maternal milk (6).

EGF and HB-EGF in developing gut

Epidermal growth factor (EGF) was first isolated from mouse salivary glands and recognized by its ability to accelerate the eruption of mouse teeth and the opening of eyelids of newborn mice (9). Human EGF was initially detected in urine (10) and then measured in many tissues and body fluids (11). EGF is a heat- and acid-stable peptide that produces a variety of biologic responses, most of which involve regulation of cell replication, cell movement and cell survival (12). In the GI tract, EGF enhances proliferation and differentiation of epithelial cells, but also has significant effects on healing of damaged mucosa or on intestinal adaptation after injury (13-16). Fetal intestine is exposed to EGF in amniotic fluid. Amniotic fluid contains significant concentrations of EGF that gradually increase during pregnancy, with the highest level achieved at the end of the normal gestation period (17). In the postnatal period, the major sources of intestinal EGF are maternal colostrum and milk (18-23). Human milk EGF levels are the highest in the first days after parturition (approximately 100 ng/mL) and then gradually decrease during the first month of lactation (20,23). Interestingly, EGF levels in milk of mothers with extremely pre-term neonates are 50-80% higher compared with those in milk of mothers with full-term infants (23). Although the physiologic relevance of this observation is still not fully understood, elevated levels of EGF in human milk may be responsible for the protective effect of maternal milk against neonatal intestinal diseases, such as necrotizing enterocolitis (NEC).

Heparin-binding epidermal growth factor-like growth factor (HB-EGF) is a member of the EGF family of growth factors. HB-EGF was initially identified in conditioned medium of macrophage-like cells as a mitogen for fibroblasts and smooth muscle cells (24). The presence of HB-EGF has been reported in both human amniotic fluid and milk (25). Several studies have shown the protective effects of HB-EGF against injury in the small intestine of adult animals (26,27). HB-EGF protects intestinal epithelium from hypoxic necrosis and cytokine-induced apoptosis and exerts its cytoprotective effects, in part via decreased nitrogen and oxygen reactive species production (26,28,29). Recent studies demonstrate the protective effects of HB-EGF against intestinal injury in the developing intestine (30-32). Compared with EGF, concentrations of HB-EGF in human milk are approximately 1,000 – 10,000 times lower (25).

However, both EGF and HB-EGF are absent from all commercially available infant formulas (33). Consequently, the developing gastrointestinal tract of prematurely born and never maternal milk-fed neonates likely misses the opportunity for long-term exposure (in the late fetal and the early postnatal period) to these two biologically active factors. This could explain the higher susceptibility to intestinal injury, such as neonatal NEC.

EGF-R and developing gut

The biological actions of EGF and HB-EGF are mediated via interaction with the EGF-R family of receptor tyrosine kinases, which includes four members, EGF-R, ErbB-2, ErbB-3, and ErbB-4 (34-36). Although EGF activates predominantly EGF-R receptor complexes (34), HB-EGF is capable of binding to and activating two tyrosine kinase receptors, EGF-R and ErbB-4 (37,38). The EGF-R is detected throughout the fetal and neonatal gastrointestinal tract (39). Under normal physiologic condition, the EGF-R is localized on the basolateral membrane of intestinal epithelial cells (40,41). However, in pathophysiologic situations such as NEC, the EGF-R is observed on both the apical and basolateral membrane of intestinal epithelium (42).

The importance of EGF-R signaling for the developing intestine is underscored by the fact that majority of the EGF-R knockout mice die during the neonatal period as a result of hemorrhagic enteritis similar to human NEC (43).

Neonatal necrotizing enterocolitis

NEC is a common and devastating gastrointestinal disease predominantly of prematurely born infants. The disease can be mild to severe with clinical presentation ranging from abdominal distension and tenderness, pneumatosis intestinalis, occult or frank blood in stools, intestinal gangrene, bowel perforation, sepsis and shock (44). Severe, end-stage NEC is characterized by an extensive hemorrhagic inflammatory necrosis of the distal ileum and proximal colon (45). This disease affects thousands of newborns every year with death occurring in up to 40% of affected individuals (44,46). Survivors of a severe episode of NEC frequently suffer the effects of short bowel syndrome (47), resulting in chronic gastrointestinal difficulties. The etiology of this disease remains poorly understood; however, intestinal immaturity, enteral feeding of infant formula, and abnormal bacterial colonization are the major risk factors to develop NEC (48,49). Currently, no predictive diagnostic tests are available and there is no effective treatment of this disease

Models of NEC and their relevance to human NEC

Several animal models have been developed to study the pathogenesis of NEC including pigs (50,51), quails (52), mice (53,54), and rats (55-57). Among these animal models, the neonatal rat (55,58) and mouse (53,54) NEC models are the most frequently used experimental model to study pathogenesis and prevention of NEC. In these models, animals are delivered by caesarian section, kept in non-sterile conditions, hand fed with cow's milk-based formula, and exposure to asphyxia and cold stress (53,59). Thus, murine NEC models incorporate all major risk factors for human NEC – prematurity of the gastrointestinal tract, enteral formula feeding, hypoxia/ischemia, and the presence of intestinal microbiota. The major advantage of murine NEC models is that many clinical and pathological changes are similar to those found in humans: the abdomen is distended, blood is detected in stool, and the ileum and proximal colon are the most affected parts of the intestine (42,53,54,57). Breast milk has been shown to have an advantage over infant formula in the reduction of human NEC (3,60-62) and maternal milk (rat milk) reduces the severity of experimental NEC in the neonatal rat model of NEC (63).

EGF in NEC

The first study relating EGF and NEC was reported in England (64). A critically ill 8-months-old child with intestinal necrosis similar to neonatal NEC was treated with continuous infusion of EGF. Within 4 days of treatment, the severely damaged intestinal structure recovered and healed (64). An initial clinical study conducted by Warner's group showed a significant reduction of salivary and serum EGF levels in premature infants with NEC compared with gestational and postnatal age-matched controls (65,66). A recent clinical trial with 327 premature and term neonates evaluated the ontogeny of salivary EGF (sEGF) and its relation to the development of NEC (67). The concentration of sEGF positively correlated with gestation age; infants at earlier gestational ages had significantly lower EGF levels in their saliva compared with term-born neonates. Importantly, lower levels of sEGF during the first week of life were associated with an increased incidence of NEC (67). These clinical findings suggest that defect in EGF plays an important role in pathogenesis of neonatal NEC, and points to consideration of EGF as an effective means to prevent or treat this disease (68).

Several lines of experimental evidence implicate an important connection between EGF and NEC. Our laboratory has previously shown that enteral administration of EGF reduces the incidence of NEC in a neonatal rat model (42). Supplementation of EGF into cow's milk-based

formula resulted in a dramatic 50% reduction in the incidence of NEC. Enhanced expression of EGF-R on both the basolateral and apical membranes in ileal epithelium of rats with NEC suggested that EGF could effectively reach the EGF-R from the luminal side (31,42). EGF-mediated protection against NEC was associated with down-regulation of pro-inflammatory IL-18 and increased production of anti-inflammatory IL-10 in the ileum - the site of injury (69,70).

The molecular mechanisms underlying EGF-mediated protection against NEC have been linked to the well-described role of EGF in altering the balance of pro-apoptotic and anti-apoptotic proteins. Supplementation of formula with EGF reduced intestinal epithelial cell apoptosis in the ileum (71). Perturbation of the intestinal barrier has been implicated in the pathogenesis of NEC (45,72-74). A recent study from our laboratory has shown that intestinal permeability, ileal goblet cell density, and mucin production are altered in rats with NEC (59). In addition to changes in epithelial cell homeostasis, oral administration of EGF decreased intestinal permeability, increased mucin production by goblet cells, and improved intestinal structure (59). All of these changes resulted in the improvement of gut integrity and enhancement of intestinal barrier function.

HB-EGF in NEC

The ability of HB-EGF to bind to and activate both EGF-R and ErbB-4 receptors led to speculation that HB-EGF could be more efficient for the treatment of intestinal injury compared with EGF, which binds only to the EGF-R (75). An initial report from our laboratory showed that HB-EGF reduces the incidence of experimental NEC (30). A similar protective effect of HB-EGF against NEC-like injury has been reported from another laboratory (32). Supplementation of HB-EGF into milk formula increased the survival rate and reduced the incidence of experimental NEC. However, doses of HB-EGF used in this study were approximately 4-5 orders higher compared with the normal physiological levels of HB-EGF in maternal milk (75). A recent report from our laboratory compared the efficacy of EGF and/or HB-EGF treatment against experimental NEC. Oral administration of either EGF or HB-EGF significantly reduced the incidence of NEC, however, EGF provided better protection in physiologically relevant doses (31). Simultaneous administration of both growth factors did not result in any additional protective effect against NEC (31). Another study comparing the efficacy of pharmacological doses of human EGF and HB-EGF against lipopolysaccharide-induced NEC-like injury in rats suggested a higher efficiency of EGF against the most severe form of this injury compared with HB-EGF (76).

Although the majority of possible molecular mechanisms responsible for EGF or HB-EGF protective effects on intestinal injury are very similar, the balance of apoptotic proteins in the ileum was shifted in favor of cell survival in EGF treated rats only (31). This may be a mechanism responsible for the higher efficiency of EGF protection against NEC. Experimental data from our laboratory indicate that physiological doses of EGF or pharmacological doses of HB-EGF could be used for prevention of NEC (31).

Conclusion

In summary, milk-borne EGF and HB-EGF play an important role in gut development during the early postanatal period. Supplementation of enteral feeds with biologically active substances normally present in breast milk seems to be a next logical step for the prevention or treatment of NEC (77). Further clinical and experimental studies are necessary before EGF can be introduced as an efficient therapeutic approach of intestinal injury.

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