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• *BRIEF REPORTS* •

Dynamic change of epidermal growth factor in neonatal rat with intestine injury

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Abstract

AIM: To determine whether diminished levels of epidermal growth factor (EGF) were present in neo-natal rats with intestinal injury and related with the degree of intestinal injury, so we modeled a model in neo-natal rats of intestinal injury and to examine the dynamic levels of EGF on injury of intestine.

METHODS: One-day-old Wistar rat pups received an intraperitoneally injection with 4 mg/kg lipopolysaccharide (LPS), followed by collection of ileum tissue at 1, 3, 6, 12, and 24 h following LPS administration. The ileum was for histological evaluation of NEC and for measurements of EGF using ABC-ELISA. The correlation between the degree of intestinal injury and levels of EGF was determined.

RESULTS: The LPS-injected pups also showed a significant increase in injury scores at 1, 3, 6, 12, and 24 h [respectively, (1.08±0.61), (1.63±0.84), (1.95±0.72), (2.42±0.43) and (2.21±0.53)] *vs* the control (0.12±0.17) (P<0.01). EGF levels at 1, 3, 6, 12 h [respectively, (245.6±49.0), (221.4±39.0), (223.4±48.1), (246.0±46.6)] pg/mg were significantly loss than the control (275.6±50.4) pg/mg (P<0.05). There was a significant negative correlation between the EGF levels and the grade of intestinal injury within 24 h (P<0.05).

CONCLUSION: Neo-natal rats with intestinal injury have significantly lower levels of ileum EGF. Reduced levels of this growth factor might be related to the pathogenesis of NEC.

Key words: Epidermal growth factor; Necrotizing enterocolitis; Rat; Newborn

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INTRODUCTION

Necrotizing enterocolitis (NEC) is a common and devastating gastrointestinal condition of premature infants[1]. The etiology of NEC appears to be multifactorial, with prematurity, enteral feeding of formula, intestinal hypoxia/ischemia, and bacterial colonization recognized as major risk factors[2,3]. These components probably act in concert to upset an already immature and delicate intestinal mucosal barrier[4]. In spite of extensive epidemiological, clinical, and basic research, the pathogenesis of NEC is not completely understood and there is no effective preventative treatment for this disease[5].

Epidermal growth factor (EGF) is a heat stable 53-amino acid peptide, which is secreted into the gut lumen by the Salivary glands, Brunner's glands of the duodenum, kidneys and also by the ulcer-associated cell lineage (UACL)[6,7]. EGF has both trophic and maturational effects on intestinal mucosal[8,9]. Previous studies suggest an important role for EGF insufficiency in the pathogenesis of NEC. These include decreased salivary and serum EGF levels in premature infants with NEC[10,11], gastrointestinal abnormalities that closely resemble NEC in EGF receptor (EGFR) knockout mice[12] and successful treatment with intravenous EGF of an 8-mo-old child diagnosed with NEC-like symptoms[13]. Moreover, Dvorak *et al*[14], established that supplementation of enteral formula feedings with EGF dramatically decreases the development and severity of NEC-like injury in the neonatal rat model. These results led us to ask whether reduced EGF might be associated with NEC in human preterm infants.

The aim of the present study was thus to determine the dynamic change of EGF in neo-natal rat with intestine injury, and to define whether NEC is associated with the levels EGF from the mucosa of the affected intestine tissue.

MATERIALS AND METHODS

Animal Model

The experiment was approved by the Second Affiliated Hospital of China Medical University. One-day-old Wistar rats (mean weight, 6.25±0.77 g) were given an intraperitoneally (IP) injection of 4 mg/kg *E.coli* O_{55}:B_5 endotoxin (lipopolysaccharide (LPS); Sigma Chemical Co., St. Louis,

Mo) or similar volume saline[15]. All pups were killed respectively at 1, 3, 6, 12, and 24 h after receiving LPS IP and the control pups were killed at 1 h after saline IP. The pups were excluded from the study were those that died before collection of the specimens.

NEC evaluation

After the rats were killed, the gastrointestinal tract was carefully removed, a 2-cm segment of distal ileum 4-cm proximal to the ileocecal valve from each animal was fixed in 4% parafarm, paraffin-embedded, microtome-sectioned at 5 μm, and stained with hematoxylin and eosin for histologic evaluation of NEC. Lesions were graded by a blinded observer and were assigned a NEC score on a scale of 0-4 as follows: 0 = normal, intact villous epithelium with normal histology; 1 = mild villous edema, with epithelial sloughing confined to the tips of the villi; 2 = mild midvillous necrosis; 3 = moderate midvillous necrosis, with crypts still readily detectable; and 4 = severe necrosis of entire villi with complete absence of epithelial structures[16].

Epidermal growth factor assay

At the time of death, the rest of the ileum from the control and LPS-injected neo-natal rats was rapidly frozen in liquid nitrogen for measurements of EGF levels. The ileums were stored at -80 ℃ until the time of the assays. Levels of EGF in ileum tissue were determined using ABC-ELISA as provided in kit form (Biosource International Inc, Belgium). The instructions of the manufacturer were followed. The protein content in each sample was estimated according to the method of Read *et al*[17], (the kit from Nanjing Jiancheng Bioengineering Institute, China). All samples were run in duplicate by an investigator who was blinded as to LPS-injected or control groups. The results were shown as EGF pg/mg protein of ileum tissue.

Statistical analysis

All the values were expressed as the mean±SE. When P was less than 0.05, the difference was considered statistically significant. Paired-Samples t test was used to check the differences between groups. The degree of correlation was described using the Spearman's rank-correlation test. Software SPSS 11.5 For Windows was used in all statistical tests.

RESULTS

Ileal EGF levels

Forty rats (8 per time point) with intestinal injury were entered into the study and eight rats served as control. The levels of EGF were significantly lower in the LPS-injected pups at 1, 3, 6, and 12 h than in the control pups. The concentration of EGF began to decrease to the minimum by 3 h after LPS-injected. No significant difference was noted between the LPS exposure pups at 24 h and the control pups (Table 1).

Incidence and severity of NEC

Using the histologic scoring system, tissues with histologic scores 2+ or greater were designated positive for NEC. In the LPS-injected group, 50.5% (21/40) showed significant (P<0.01) pathologic changes in ileal structure characterized as moderate (2+), severe (3+), or full necrosis (4+) versus only 0% (0/8) incidence of NEC in the control group. The degree of ileal damage was also significantly (P<0.01) increased in the LPS-injected versus the control group, with a median NEC score of 1.08±0.61, 1.63±0.84, 1.95±0.72, 2.42±0.43, and 2.21±0.53, respectively. The median for NEC damage in control animals was 0.12±0.17. Spearman's rank correlation analyses showed that lower EGF concentrations correlate with increased severity of NEC (r = -0.547, P<0.01).

DISCUSSION

In the current study, we have measured substantially lower levels of EGF in the ileum tissue with complicated intestinal injury, when compared with a cohort of control rats. These findings offer a potential role for a deficiency of EGF in the initiation or progression of NEC. There was a significant negative relationship between the levels of EGF and the degree of intestinal injury. These results confirm that decreased levels of EGF of the ileum result in a dramatic augment of the incidence and severity of NEC.

NEC is the most common seriously acquired gastrointestinal tract problem in neonatal intensive care units, with reported mortality of 10-30%[18]. Furthermore, the severity of NEC, as per the modified Bell's classification as well as mortality, seems to be inversely related to birthweight and gestational age, with a report of even 100% mortality in infants with birth-weights less than 1 000 *g* and gestational ages less than 28 wk[19]. The reasons for a predilection for prematurity are unclear, but an immature mucosal barrier and immune response likely contribute to the premature neonates' susceptibility[14]. EGF is acid-stable and trypsin- resistant, allowing it to survive passage through the gastrointestinal tract and to act directly on the intestinal cells[20]. The predominant effects of EGF on the gastrointestinal tract have been reported and include suppression of gastric acid secretion, gastric cytoprotection; stimulation of intestinal DNA synthesis and cell division; regulation of intestinal brush-border disaccharidase activities; increased water, glucose, and sodium absorption; increased cellular calcium concentration; activation of ion transport and modulation of prostaglandin synthesis and secretion [6,21-23]. These studies

Table 1 EGF levels of ileum of neonatal rats (n = 8, pg/mg)

	Control group	LPS group				
		1 h	3 h	6 h	12 h	24 h
EGF	275.6±50.4	245.6±49.0[a]	221.4±39.0[b]	223.4±48.1[b]	246.0±46.6[b]	240.8±34.6

[a]P<0.05, [b]P<0.01 *vs* compared with control group, respectively.

suggested that EGF plays an important role in fetal or postnatal intestinal growth and development.

Experimental evidence supports a role for EGF on gut maturation and protection during development; EGF leads to increased growth of the gastric mucosa in neonatal rats, and inactivation of the EGFR in knockout mice results in a hemorrhagic enteritis that is similar to NEC[12,24-26].

The first report relating EGF and intestinal necrosis was reported in England, where an 8-mo-old child with intestinal necrosis similar to NEC, that serial biopsies showed significant increase in crypt cell proliferative activity in association with marked recovery of the surface epithelium, was successfully treated with continuous infusion of EGF[13]. Scott *et al*[27], have shown significant elevation of urinary EGF levels in neonates at the time of the development of NEC that they suggest might result from increased absorption of EGF through the damaged intestinal mucosal. Furthermore, markedly diminished serum and salivary EGF levels have been reported in premature infants with NEC[10,11]. These studies suggested that administration of exogenous EGF might provide an effective means to prevent or treat this disease. Therefore, supplementation of a higher dose of EGF into milk formula can protect EGF against this inherent proteolytic degradation in the stomach and small intestine and improve the efficiency of injury treatment during the perinatal period of life[14].

The mechanisms underlying the protective effects of EGF in injured mucosal are not clearly understood. However, the involvement of the EGFR in the biological action of EGF has been extensively studied[28]. Various studies in the past have described the appearance of EGFR in the human digestive tract between the 12th and 17th wk of gestation[29-31]. EGFRs are present throughout the gastrointestinal tract on the basolateral membrane, which may be more accessible in the preterm intestine with increased permeability[30,31]. It promotes intestinal growth and stimulates intestinal repair[12,31,32]. The EGFR is activated through phosphorylation by prostaglandin PGE_2 producing a large number of downstream events that can stimulate growth-related signal transduction to regulate the cell cycle and enhance cell proliferation[33-36].

Our present study provides, for the first time, the decreased levels of EGF in damaged ileum indicate that offers a potential role for a deficiency of EGF in the initiation or progression of NEC. Moreover, the development of NEC has effect on the endogenous production of EGF in damaged ileum.

In conclusion, the degree of NEC is associated with the decreased levels of EGF that may support the possibility of using exogenous administration of EGF for prophylaxis or treatment of this condition.

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