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# Expression of epidermal growth factor and its receptor in the gastric mucosa of neonatal rats with intrauterine asphyxia

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**Abstract: Objective** Neonatal asphyxia may induce acute gastrointestinal injuries such as erosion, ulcer, bleeding and so on. Recent investigations have shown that epidermal growth factor (EGF) and its receptor (EGFR) play an important role while participating in the reparation of acute gastrointestinal injury in adult animals. This paper aims at studying the expressions of EGF and EGFR in gastric mucosa of neonatal rats with intrauterine asphyxia as well as their roles in the reparation of gastric mucosa lesions. **Methods** Wistar rats, 21 days pregnant, were used to establish animal models of intrauterine asphyxia. The surviring pups of the Asphyxia group and the pups without asphyxia (Control group) were sacrificed by decapitation at 0, 24, 48, 72 hrs after birth and gaster specimens were collected. The expressions of EGF, EGFR and EGFR mRNA in the gastric mucosas were assayed by immunohistochemistry and reverse transcription-polymerase chain reaction (RT-PCR) and the histology of gastric mucosas were observed. **Results** (1) The gastric mucosa lesions of neonatal rats were found in the Asphyxia group and became more and more serious untill a peak was reached 48 hrs after birth. (2) Low expressions of EGF and EGFR could be detected in the gastric mucosas of rats in the Control group, and there were no differences among various time points. (3) The expressions of EGF and EGFR in the gastric mucosas of neonatal rats in the Asphyxia group increased with time and reached the peak 48 hrs after birth. The EGFR expressions at 24, 48 and 72 hrs in the Asphyxia group were significantly higher than those of the Control group ($P < 0.01$ or $0.05$). (4) The expression of EGFR mRNA in the gastric mucosa of neonatal rats in the Asphyxia group reached a peak 24 hrs after birth and was higher than that of the Control group ($P < 0.01$). **Conclusions** EGF and EGFR may play an important role in the reparation of gastric mucosa lesions of neonatal rats with intrauterine asphyxia.

**[Chin J Contemp Pediatr, 2004, 6(1): 7 - 10]**

**Key words:** Asphyxia; Gastric mucosa; Epidermal growth factor (EGF); Epidermal growth factor receptor (EGFR); Neonatal rat

## 宫内窒息后新生鼠胃粘膜表皮生长因子及其受体的研究

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[**摘 要**] **目的** 新生儿窒息可引起糜烂、溃疡和出血等胃肠粘膜损伤。既往成年动物研究发现表皮生长因子(EGF)及其受体(EGFR)在急性胃肠粘膜损伤后的修复过程中发挥重要作用。该文旨在探讨 EGF 和 EGFR 在宫内窒息新生鼠胃粘膜的表达及其在胃粘膜损伤后修复过程中的作用。**方法** 用孕足 21 d 大鼠制作成宫内窒息模型,将剖宫产后存活的窒息组和对照组新生鼠在出生 0、24、48 和 72 h 断头处死,留取胃标本。应用免疫组化方法和反转录 - 多聚酶链反应动态监测新生鼠胃粘膜 EGF、EGFR 和 EGFR mRNA 的表达,同时观察胃组织形态学改变。**结果** (1)窒息组新生鼠出现胃粘膜损伤,于生后 48 h 损伤最重,之后开始恢复。(2)对照组新生鼠胃粘膜有较弱的 EGF 和 EGFR 表达,各时间点差异无显著性($P > 0.05$)。(3)窒息组新生鼠胃粘膜 EGF 和 EGFR 的表

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达随生存时间增加而增强,在 48 h 达高峰之后开始下降,生后 24,48,72 h 胃粘膜 EGF 表达与对照组同时相比较,差异均有显著性( $P < 0.01$ 或 0.05)。(4)窒息组新生鼠胃粘膜 EGFR mRNA 的表达于 24 h 达高峰,较对照组同时间点相比增加,差异非常显著性( $P < 0.01$),之后开始减少。**结论** EGF 和 EGFR 可能在窒息后新生鼠胃粘膜损伤后的修复过程中发挥重要作用。 [中国当代儿科杂志,2004, 6(1): 7 - 10]

[关 键 词] 窒息;胃粘膜;表皮生长因子;表皮生长因子受体;新生鼠
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It has been found that the blood flow of celiac trunk artery decreases by 70 % after asphxia[1], which causes the blood flow of gastrointestinal tract to drop off and leads to gastrointestinal mucosa lesions, such as erosion, ulcer, bleeding and even necrotizing enterocolitis (NEC). Recent investigations have shown that epidermal growth factor (EGF) participated in reparative course of acute gastrointestinal lesions in the adult animals[2]. EGF and its receptor (epidermal growth factor receptor, EGFR) appeared to play important roles in the development of gastrointestinal tract, inhibition of gastric acid secretion, protecting gastrointestinal tract and promotion of gastrointestinal lesions healing. However, there has been no report on the expressions of EGF and EGFR in the gastrointestinal mucosa of neonates or neonatal rats with intrauterine asphyxia. This present study explores the expressions of EGF and EGFR in the gastric mucosa and their roles in the reparation of gastric mucosa lesions in neonatal rats with intrauterine asphyxia.

## Materials and methods

### Animals and grouping

Sixty-three healthy Wistar rats (53 female and 10 male) were provided by the Animals Laboratory of the Second Clinical Hospital of China Medical University. The average weights of female and male rats were 270 ±30 g and 300 ±20 g, respectively.

The animal model of intrauterine asphyxia was established according to the method provided by Tanaka[3]. The Wistar rats which were 21 days pregnant were anesthetized with ether. Two-horn uteri and vessels supplying the uteru and ovaries were exposed and one side of the vessel was occluded with arterial clamps. The fetus rats in the occluded side of the uteru were used as the Asphyxia group and ones in the other side were used as the Control group. After 20 minutes of blockage of the blood flow the uteri were opened and the pups were taken out. The surviving neonatal rats in the Asphyxia group and the pups of the Control group were sacrificed at 0, 24, 48 and 72 hours (15 - 20 rats at each time point).

### Reagents

The immunohistochemistry reagents of EGF and EGFR were obtained from Zhongshan Biological Limited Company (Beijing). The total RNA extraction system was provided by Huamei Biological Limited Company (Luoyang, Henan). PCR primers were synthesized by Aoke Biological Technological Company (Beijing). The reverse transcription reagents were from Takara Company.

### Disposal of specimens

Three gaster specimens of each group at each time point were used to observe histological changes of gastric mucosas, another 3 were preserved with 10 % formalin for detection of expressions of EGF and EGFR, and another 5 were kept at - 80 ℃ in eppendorf tubes without RNAase to assay EGFR mRNA expression.

### Observation of gastric mucosa histology

After dehydration, the gastric tissues were embedded in paraffin blocks and sliced into 4 μm thickness and then they were dewaxed and stained with hematoxylin (HE).

### Measurement of EGF and EGFR expressions

The average gray density of EGF and EGFR were measured with Meta Morph software. The expressions of EGF and EGFR were assayed by strept Avidin complex (SABC) and the detailed procedures were carried out according to the directions of the reagents. The images of 6 visual fields in each section were extracted with PLYMPUS - BX51 photograph extraction system (Japan).

### Measurement of EGFR mRNA expression

The procedure was performed following the directions and the relative contents of EGF mRNA were obtained.

**Statistical analysis**

All data were expressed as $\bar{x} \pm s$ and $q$ test were used to analyze the differences.

## Results

**Histological changes of the gastric mucosas**

The histological changes of gastric mucosas at 0 hour in the Asphyxia group were not different from those of the Control group. The epithelial cells of the gastric mucosas swelled and attached a little inflammatory exudation at 24 hours in the Asphyxia group. The epithelial cells developed degeneration, some of them even exfoliated, and infiltrations of lymphocytes, plasmacytes and a few neutrophilic granulocytes could be found in intestitium at 48 hours. These lesions of gastric mucosas relieved at 72 hours. There were no these histological changes in the gastric mucosas of the Control group.

**EGF expression in the gastric mucosas**

Low EGF expression in the gastric mucosas of the Control group was found in cytoplasm of epithelial cells but there were no differences among various time points. These expressions mainly located in the proliferative zone of the gastric mucosas, chief cells, parietal cells, and basal of gastric glands. Compared with the Control group, the EGF expression in the gastric mucosa of the Asphyxia group at 0 h were not different. It increased at 24 hours, reached the peak at 48 hours and remained higher at 72 hours than those of the Control group ($P < 0.01$ or 0.05) (Table 1, Figure 1, and Figure 2).

**Table 1** EGF expression in gastric mucosa ($n = 3$, $\bar{x} \pm s$)

| Group | 0 hr | 24 hrs | 48 hrs | 72 hrs |
|---|---|---|---|---|
| Control | 141.487 ±0.907 | 141.429 ±1.310 | 141.834 ±0.999 | 141.772 ±0.753 |
| Asphyxia | 141.310 ±0.821 | 142.256 ±0.885[a] | 143.118 ±1.142[b,c] | 142.383 ±0.808[a,c] |

Note: a vs Control group $P < 0.05$; b vs the Control group $P < 0.01$; c vs the previous time point in the Asphyxia group $P < 0.05$

**EGFR expression in the gastric mucosas**

Low EGFR expression in the gastric mucosas of the Control group was found in membrane of epithelial cells. These expressions were mainly located in the proliferative zone of gastric mucosa, chief cells, parietal cells and the basal of the gastric glands. The expressions at various time points were not significantly different. The expressions of EGFR in the gastric mucosas in the Asphyxia group at 0 hour was significantly lower than that of the Control group ($P < 0.01$), while no significant differences were found at 24 hours between the two groups. The EGFR expressions in the Asphyxia group reached a peak at 48 hours and were much higher than that of the Control group ($P < 0.01$). The expression of EGFR at 72 hours in the Asphyxia group were not significantly different from those of 24 and 48 hours following asphyxia although it was higher than that of the Control group ($P < 0.05$) (Table 2, Figure 3, and Figure 4).

**Table 2** EGFR expression in gastric mucosas ($n = 3$, $\bar{x} \pm s$)

| Group | 0 hr | 24 hrs | 48 hrs | 72 hrs |
|---|---|---|---|---|
| Control | 107.326 ±4.119 | 107.591 ±5.907 | 105.306 ±6.946 | 105.874 ±6.188 |
| Asphyxia | 95.802 ±6.465[a] | 106.084 ±7.538 | 112.143 ±7.086[a,c] | 110.012 ±5.338[b] |

Note: a vs the Control group $P < 0.01$; b vs the Control group $P < 0.05$; c vs 24 hrs in the Asphyxia group $P < 0.05$

**EGFR mRNA expression in the gastric mucosas**

The EGFR mRNA expressions in the gastric mucosas in the Control group at various time points were not significantly different. The expression of EGFR mRNA in the gastric mucosas in the Asphyxia group at 0 hour were significantly lower than that of the Control group ($P < 0.01$). After 24, 48 and 72 hours of asphyxia the expressions of EGFR mRNA of

the gastric mucosas were increased significantly compared with those of the Control group ( $P < 0.01$). There were significant differences in the EGFR mRNA expression between 24 hours and 48 hours of asphyxia and between 24 hours and 72 hours of asphyxia ( $P < 0.01$). (Table 3, Figure 5, and Figure 6)

**Table 3** EGFR mRNA expression in gastric mucosas ($n = 5$, $\bar{x} \pm s$)

| Groups | 0 hr | 24 hrs | 48 hrs | 72 hrs |
|---|---|---|---|---|
| Control | 0.736 ±0.031 | 0.708 ±0.019 | 0.704 ±0.011 | 0.718 ±0.022 |
| Asphyxia | 0.574 ±0.011[a] | 1.204 ±0.021[a] | 0.820 ±0.016[a,b] | 0.800 ±0.016[a,b] |

Note: a vs the Control group $P < 0.01$; b vs 24 h in the Asphyxia group $P < 0.01$

## Discussion

EGF, a potent mitogen, exists in gastrointestinal mucosa, lattices, saliva, gastric and duodenal juice, pancreatic juice and bile and other tissues and body fluid. It plays an important role in promoting proliferation and differentiation of epithelial cells, development of fetus, renovation and regeneration of tissues[4]. EGFR is a 170 KD protein that consists of a cell surface ligand binding domain and exists in gastrointestinal epithelium[5]. The combination of EGF and EGFR can result in clustering, dimerisation and internalization of the receptor-ligand and induce a series of responses: increasing production of mucoprotein, stimulating migration of epithelial cells, synthesis of DNA, RNA and protein and division of cells.

Previous studies[6] have shown that EGF and EGFR could stimulate restitution and proliferation of gastrointestinal mucosa cells, inhibit secretion of gastric fluid, increase blood flow of gastrointestinal mucosa and promote healing of acute and chronic gastrointestinal injuries. In this study, epithelia cells edema and exfoliation and infiltrations of inflammatory cells in gastric mucosa were found in neonatal rats with asphyxia. These changes became more and more serious during the course and reached the peak 48 hours after birth, and then began to restore. The expressions of EGF and EGFR in gastric mucosa of neonatal rats with asphyxia increased with time and reached the highest level 48 hours after birth and then began to decrease gradually. The EGFR mRNA expressions in the gastric mucosa of neonatal rats with asphyxia reached the peak 24 hours after birth, and then began to decrease gradually. The increase of both EGF expression and EGFR expression resulted in an increase of blood flow of the gastric mucosa and proliferations of mucosa cells as a result of promoting healing of acute gastric mucosal injuries. This suggested that EGF and EGFR play an important role in the reparation of gastric mucosal lesions in neonatal rats with asphyxia.

Furthermore, recent foreign investigations[5] have shown that pretreatment with EGF can decrease the incidence and severity of acute and chronic gastrointestinal injury. These studies provide a new potential approach for the prevention and treatment of gastrointestinal mucosa lesions.

(Figures are on the inside front cover)

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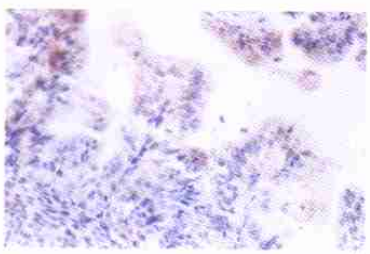
# Expression of epidermal growth factor and its receptor in the gastric mucosa of neonatal rats with intrauterine asphyxia

( These figures refer to the paper on page 7 )



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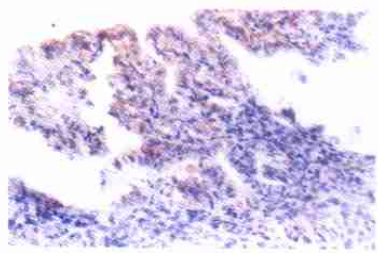

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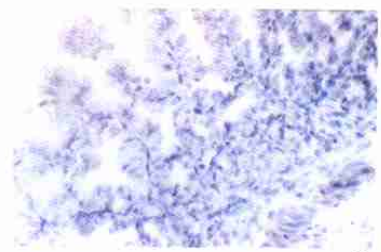

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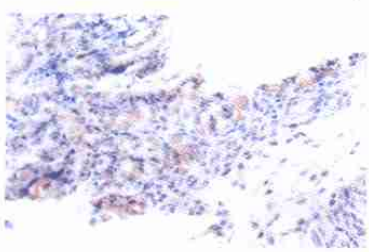

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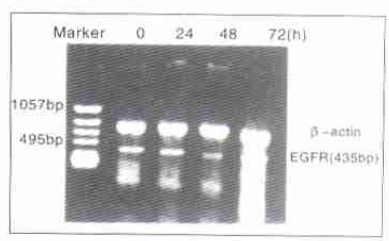


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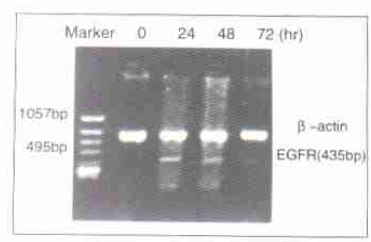


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**Figure 1** EGF expression of the gastric mucosa in the Control group. Cytoplasm of epithelial cells were light brown after having been stained (400 ×)

**Figure 2** EGF expression of the gastric mucosa 20 minutes afer asphyxia. Cytoplasm of epithelial cells were dark brown after having been stained (400 ×)

**Figure 3** EGFR expression of the gastric mucosa in the Control group. Membrane of epithelial cells were light brown after having been stained (400 ×)

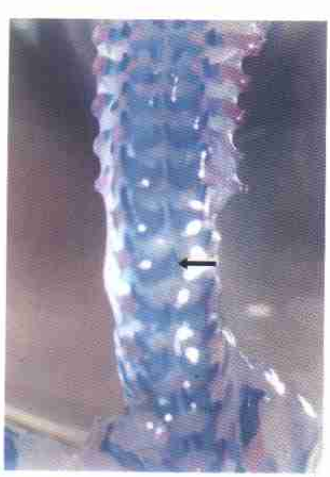
**Figure 4** EGFR expression of the gastric mucosa 20 minutes after asphyxia. Membrane of epithelial cells were dark brown having been stained (400 ×)

**Figure 5** EGFRmRNA expression of the gastric mucosa at various time points in the Control group. No difference among various time points was found (400 ×)

**Figure 6** EGFR mRNA expression of the gastric mucosa at various time points in the Asphyxia group. The lowest expression was at 0 hour and the highest was at 24 hours (400 ×)

# 丙戊酸诱导胎鼠发生隐性脊椎裂畸形的模型建立

（正文见第38页）



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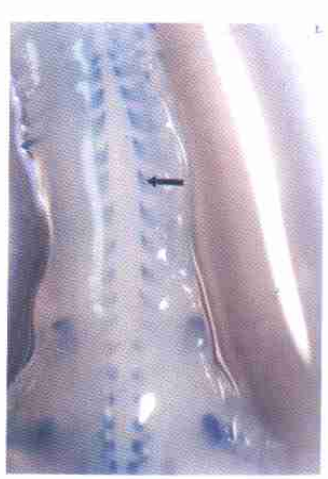

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图1　正常胎鼠20天，脊柱双重染色，椎体椎弓两个软骨端距离非常接近，从胸9到腰6距离均小于相应椎体正常范围值，箭头示腰2距离值为104.1 μm。（7.5倍×2.5倍）

**Figure 1** The distances between the ends of the vertebral archs from T9 to L6 were less than the normal limit. The distance of L2 (arrow) was 104.1 μm.(7.5×2.5) (spina double-staining in the normal fetal rats)

图2　脊柱椎体软骨被阿尔新蓝染成蓝色后可见胎鼠脊椎从胸9到骶5椎体弓两个软骨端距离都增大，出现了隐性脊椎裂；箭头示腰2距离最大611.9 μm。（7.5倍×2.5倍）

**Figure 2** After being stained, the distances between the ends of the vertebral archs from T9 to S5 were shown broad in the VPA group. The distance of L1 (arrow) was the most broad (611.9 μm).(7.5 × 2.5)