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Effect of Asphyxia on Urinary Epidermal Growth Factor Levels in Newborns

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Summary: Urinary epidermal growth factor (EGF) excretion in normal newborn as well as neonates with asphyxia was investigated by using radioimmunoassay, and serum creatinine (Scr) levels determined at the same time. The results showed that in severe asphyxia group the ratio of urinary EGF to urinary creatinine (Cr) (EGF/Cr), an index reflecting EGF excretion, was decreased on the first day ($P<0.05$) and reached the lowest level on the third day ($P<0.01$). However, EGF/Cr values were decreased only on the third day in neonates with mild asphyxia ($P<0.05$). On the seventh day, EGF/Cr values of neonates with asphyxia rose to normal. There were a negative correlation between urinary EGF/Cr and Scr. It is suggested that EGF may play a role in the repair of acute renal injury after asphyxia and the detection of urinary EGF concentration is useful in the judgment of severity of renal injury and in the evaluation of the recovery of renal tubule after injury.

Key words: asphyxia; newborn; renal injury; epidermal growth factor

Epidermal growth factor (EGF) is a potent polypeptide mitogen that can be isolated from all body liquids, secretion and many tissues. Kidney is one of main sites of EGF synthesis. EGF has been demonstrated to stimulate a variety of biological responses *in vitro*, including modulation of cellular growth and metabolism, regulation of glomerular hemodynamics, promotion of Na^+/H^+ exchange in renal tubule, acceleration of renal tubular epithelial cell regenerative and repairing processes after renal injury[1]. Urine contains high concentration of EGF, which is thought to be of renal origin. Despite the fact that renal prepro-EGF mRNA and urinary EGF excretion have been found to be decreased during ARF in the rat[2], the effect of asphyxia on urinary EGF excretion *in vivo* remains undefined. The aim of this study was to understand the changes of urinary EGF after ischemic injury and the relationship between these change and renal functional injury.

1 PATIENTS AND METHODS

1.1 Subjects and Samples

Twenty neonates with asphyxia were divided into two groups in terms of degree of asphyxia. Apgar scores in mild asphyxia group (7 cases) was 4 to 7 and 1 to 3 in severe asphyxia group (13 cases). The gestational age of these infants ranged from 36 to 42 weeks, birth weight from 2 400 to 3 900 g (14 males and 6 female). Nine health neonates served as controls. No obvious difference in gestational age, gender and birth weight between asphyxia and control groups was found.

Urine samples were randomly obtained for analysis of urinary EGF and creatinine (Cr) on the first, third and seventh day after birth and blood specimens were obtained for detection of serum creatinine (Scr) on the second day from all subjects.

1.2 EGF Assay and Creatinine Determination

EGF levels were determined by using radioimmunoassay. Human EGF kit was provided by Hai Ke Rui Biological Technical Center, Beijing. Antiserum (0.2 ml) and ^{125}IhEGF (0.1 ml) was added to urine aliquotes (0.1 ml) contained in a test tube and incubated for 24 h at 40 ℃. The second antibody (0.5 ml) was then added to the tubes. After incubation for 15 min at room

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temperature, the tubes were centrifuged for 15 min at 2500 r/min. The supernatants were discarded, and the tubes were counted with a gamma scintillation counter. Scr was determined with automatic chemical analyzer.

1.3 Statistical Analysis

Excretion of EGF was expressed as a ratio of urinary EGF to urinary creatinine (EGF/Cr) for all samples. Data were given as $\bar{x} \pm s$. Analysis of variance and q test were used to compare the difference of urinary EGF/Cr among different groups. Linear regression analysis was employed to examine the relationship between EGF/Cr and Scr. Statistical significance was set at $P < 0.05$.

2 RESULTS

2.1 Urinary EGF Levels in Normal Neonates

Urinary EGF levels were 68.1±23.8, 83.5±9.7 and 67.8±27.3 ng/ml on the 1st, 3rd and 7th day, respectively. Statistically, urinary EGF levels were unchanged within 7 days after birth. Urinary EGF levels of normal neonates were higher than those of the healthy adults (27.17±22.6 ng/ml, $P<0.01$)[3].

2.2 EGF/Cr in Asphyxial Neonates

Table 1 shows the dynamic changes in EGF/Cr in the three groups. The results demonstrate that there was a significant decline in EGF/Cr values in neonates with asphyxia on the 1st to 3rd days, whereas the values rose to normal at the 7th day. EGF/Cr values were significantly lower in severe asphyxia group than those in control group on 1st day (70.6±41.8 versus 137.5±32.0 ng/mg, $P < 0.05$). The lowest EGF/Cr was found on 3rd day. EGF/Cr values in severe or mild asphyxia group were significantly lower as compared with those of control group (65.1±37.6 versus 190.2±80.6, $P<0.01$, 113.3±41.1 versus 190.2±80.6, $P<0.05$). EGF/Cr values on 7th day revealed no statistical difference between two asphyxia groups and control group.

2.3 EGF/Cr vs Scr

Our results demonstrated a highly significant negative correlation between urinary EGF/Cr values and Scr concentrations in severe or mild asphyxia groups ($r = -0.793$, -0.754, $P<0.001$, table 2).

Table 1 EGF/Cr values in normal and asphyxiate newborns ($\bar{x}\pm s$ ng/mg)

Group (n)	1st d	3rd d	7th d
Control(9)	137.5±32.0	190.2±80.6	170.1±65.7
Mild asphyxia(13)	116.8±65.4	113.3±41.1*	143.6±97.0
Severe asphyxia(7)	70.6±41.8**	65.1±37.6**	152.7±64.9
	$F=4.62$	$F=11.63$	$F=0.19$
	$P<0.05$	$P<0.001$	$P>0.05$

*: $P<0.05$, **: $P<0.01$

Table 2 The relationship between EGF/Cr (ng/mg) and Scr (μmol/L) in newborn of asphyxia

Group (n)	EGF/Cr	Scr	r	P
Severe asphyxia(7)	65.1±37.6	91.2±48.9	−0.793	<0.001
Mild asphyxia (13)	113.3±41.1	70.9±51.3	−0.754	<0.001

3 DISCUSSION

Asphyxia of newborn may lead to injury or failure of some organs because of hypoxia and ischemia of tissues and organs. Acute renal injury was mostly frequent after asphyxia. Ischemic acute renal failure is a reversible form of organ failure. It has two phases, i. e., injury and recovery phases. The injury phase is the structural damage to renal epithelial cells, predominantly proximal tubule cells. The recovery phase is therefore dependent on the repair and re-

placement of the injured and necrotic tubule epithelial cells[4]. Recently, a series of studies have revealed that EGF was one of most potent mitogenic stimuli of renal proximal tubule cells and was a possible mediator of cellular proliferation in the kidney. EGF can increase transcriptional activity, DNA synthesis, mitosis and cellular proliferation, and it is important to the recovery of renal functions after injury[5, 6].

EGF synthesized within the kidney is thought to be the primary source of urinary EGF. Urinary EGF concentration is very low before 30th week of gestation age and rise rapidly after birth (<0.05 nmol/L in human amniotic fluid)[7]. The highest urinary EGF was found during the first month to the 3th years after birth[8]. Our studies showed that urinary EGF levels in normal neonates were significantly higher than those of healthy adults, demonstrating that EGF takes a part in renal maturing. Animal studies have demonstrated that soluble immunoreactive EGF (irEGF) content in rat kidney was increased after ischemia-induced acute renal failure, which peaked 24 h after injury and returned to normal values within 72 h. However, both renal prepro-EGF mRNA and urinary excretion of EGF were decreased after ischemia in the rats. In addition, EGF receptor density and specific EGF binding were increased[2]. Our studies revealed that EGF/Cr values in neonates with asphyxia were decreased, and the rapidity and extent of declining were consistent with severity of asphyxia. EGF/Cr values in severe asphyxiate neonates fell from 137.5±32.0 ng/mg in control group to 70.6±41.8 ng/mg on the 1st day and were further reduced to 34% of control values on the 3rd day (65.1±37.6 versus 190.2±80.6 ng/mg). In mild asphyxia group, EGF/Cr values were statistically lower than those of control group only on the 3rd day (113.3±41.1 versus 190.2±80.6 ng/mg, $P<0.05$). Subsequently, EGF/Cr values rose progressively in asphyxia groups and showed no significant difference from the control values on the 7th day ($P>0.05$). In addition, there were a negative correlation between EGF/Cr and Scr values after asphyxia, suggesting that increased EGF/Cr values may follow renal recovery from ischemic renal failure. The mechanism of reduced EGF excretion remains unknown. It was reasoned that ischemia affected both transcriptional and post-transcriptional effects of EGF and decreased renal prepro-EGF mRNA production. Meanwhile renal EGF receptor density and specific EGF binding were increased after ischemic renal injury. The up-regulation of the renal EGF receptor may augment renal EGF actions, including increased renal vasoconstriction, diminished water reabsorption, and enhanced mitogenesis to accelerate renal functional recovery[5]. Declined EGF receptor density and increased EGF excretion followed the recovery of renal tubule injury[9].

To conclude, EGF may play a role in the recovery phase of renal ischemic injury. EGF/Cr values after asphyxia may be a early parameter helpful in the evaluation of the severity of renal ischemic injury and proliferating of renal tubule injury.

A recent report[10] has documented that exogenous administration of EGF early in the recovery phase of ischemic acute renal failure could enhance renal tubule cell regeneration as well as lessen the severity and duration of acute renal failure. These offer a new way for preventing renal injury after asphyxia with exogenous EGF.

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