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Serum EGF and NGF levels of patients with brain injury and limb fracture

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ABSTRACT

Objective: To explore the expression and significance of human epidermal growth factor (EGF) and nerve growth factor (NGF) in patients with knee osteoarthritis. **Methods:** RT-PCR and enzyme-linked immunosorbent assay were used to measure the serum EGF and NGF expression levels of patients with limb fracture and brain trauma injurry after 1 d, 3 d, 7 d, 14 d and the relationship between them was analyzed. The level was compared among the simple fracture group, traumatic brain injury group and the normal control group, with 40 cases in each group. **Results:** The serum NGF levels were significantly different among three groups. Serum NGF, EGF mRNA and protein levels gradually decreased with the increasing injury time in the limb fracture combined with brain injury group, traumatic brain injury group, the simple fracture group and the health control group ($P<0.05$). **Conclusions:** The serum of NGF, EGF levels significantly increased when limb fracture combined with brain injury, so EGF and NGF may be involved in the process of fracture healing.

1. Introduction

Fracture healing is a complex and orderly process. In clinical process, the patients of limb fracture combined with brain injury have overgrowth and large number of callus, and heterotopic ossification appear even in muscle. The fracture healing process is significantly faster than patients without brain injury[1,2], but the mechanism is still unclear. With the development of molecular biology research, we have a better understanding of the molecular mechanisms of the fracture healing. The local tissues of the fractures release a variety of growth factors after a short period of the injury, and the bone and cartilage cells still produce the growth factor to repair until the late stage. These growth factors interact with each other to promote the healing of fractures. In clinic, many scholars positively seek the bridge factor of the central nervous system and bone metabolism. In this study, the changes of epidermal growth factor (EGF) and the nerve growth factor (NGF) were studied to explore the critical factor which can promote fracture healing.

2. Materials and methods

2.1. General information

Forty cases of limb fracture combined with brain injury were selected from January 2011 to October 2012, The diagnostic criteria for the fracture was proved by X-ray. The inclusion criteria for the combined traumatic brain injury included history of headache, vomiting and coma, which persist for more than three days, and CT showing significant cerebral contusion. GSM evolutions for patients of limb fracture combined with brain injury were from 6 to 12, patients´ ages were from 21 to 68 years old. The average age was 41.3±8.9, including 25 males and 15 females. The age of simple fracture group was from 22 to 70. The average age was 41.6±7.8, including 27 males and 13 females. Age of traumatic brain injury group was from 24 to 71, the average

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age was 41.9±8.0, including 28 males and 12 females. Age of healthy volunteers group was from 21 to 72. The average age was 40.6±8.4, including 24 males and 16 females. All patients with serious respiratory, cardiovascular, diabetes, bone metabolic diseases, other thoracoabdominal severe trauma osteoporosis and osteogenesis imperfecta or other foundermental diseases were excluded.

2.2. Detection method

A total of 5 mL elbow midvenous blood was collected from the enrolled patients in the early morning of 1, 3, 7 and 14 days after trauma. After blood collection, it was solidified at room temperature for 30 min, centrifuged at 3 500 rpm for 5 min. The supernatant was collected and then placed at −70 ℃ refrigerator. EGF and NGF were detected by double antibody sandwich ABC-ELISA method (Boster anti-human NGF-ELISA kit). Anti-human EGF and NGF monoclonal antibody coated the enzyme label plate was used; a biotinylated antihuman NGF antibody was added to form immune complexes and attached to the board. The horseradish peroxidase conjugated Streptavidin was combined with the biotin, and an enzyme substrate OPD was added; the OD value was proportional to the concentration of NGF. The NGF concentration was determined by the standard curve.

2.3. Statistical analysis

The experimental data were analyzed with SPSS16.0 statistical software. The difference between groups was analyzed by χ^2 test. Data were expressed as mean±SD values. The difference between two groups was analyzed with single factor analysis of variance. $P<0.05$ was regarded as statistically significant.

3. Results

The NGF and EGF levels gradually increased in limb fracture combined with brain injury group, traumatic brain injury group and simple fracture group. The serum of NGF and EGF levels at 1 d, 3 d, 7 d, 14 d after injury in limb fracture combined with brain injury group were significantly higher than those of the simple fracture group and the traumatic brain injury group ($P<0.05$). The serum of NGF and EGF levels at 1 d, 3 d, 7 d, 14 d after injury in traumatic brain injury group were significantly higher than the simple fracture group ($P<0.05$) (Table 1, Figure 1 and Figure 2).

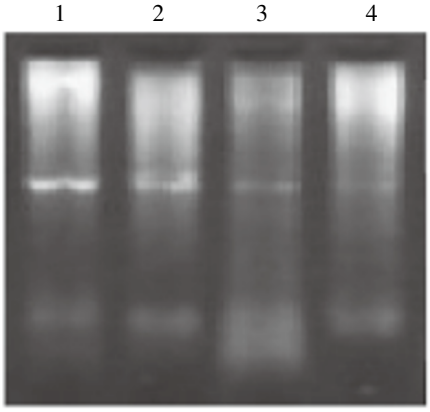

Figure 1. NGF level.
1, Limb fracture combined with brain injury group; 2, Traumatic brain injury group; 3, Simple fracture group; 4, Normal control group.

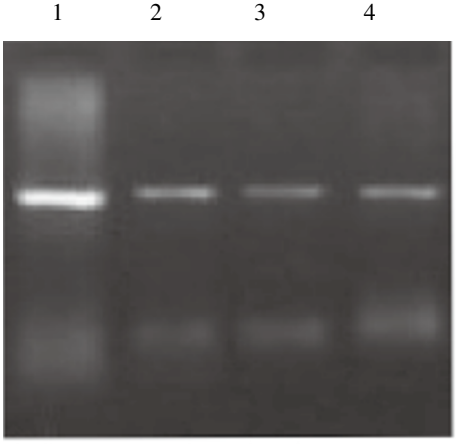

Figure 2. EGF level.
1, Limb fracture combined with brain injury group; 2, Traumatic brain injury group; 3, Simple fracture group; 4, Normal control group.

Table 1
mRNA expression levels of NGF and EGF.

Indexes		Limb fracture combined with brain injury group	Traumatic brain injury group	Simple fracture group	Normal control group
NGF mRNA expression levels	1 d	0.875±0.135	0.584±0.104	0.326±0.087	0.146±0.049
	3 d	0.926±0.149	0.631±0.110	0.368±0.092	0.149±0.048
	7 d	0.957±0.132	0.684±0.098	0.399±0.090	0.152±0.047
	14 d	0.989±0.138	0.703±0.112	0.442±0.091	0.148±0.046
EGF mRNA expression levels	1 d	0.892±0.145	0.683±0.132	0.463±0.110	0.262±0.084
	3 d	0.924±0.154	0.724±0.135	0.489±0.108	0.267±0.086
	7 d	0.963±0.162	0.763±0.127	0.532±0.115	0.265±0.088
	14 d	1.188±0.149	0.798±0.125	0.568±0.121	0.268±0.089

4. Discussion

With increaseing traffic accident, number of trauma patients increases year by year, and many of them have limb fracture combined with brain injury. Clinical study have shown that the fracture healing process of limb fracture combined with brain injury is significantly shorter than simple fracture. Often there will be more calluses at the fracture site after 2 to 3 weeks of injury, and myositis ossificans and heterotopic ossification incidence is relatively high in traumatic brain injury patients[3,4]. Studies have shown[5,6] that the damaged brain tissue or the pituitary gland secretes a large number of growth factors and inflammatory cells, they can release and secrete a variety of cytokines which promote the healing of fractures by fluid circulation and paracrine in the fracture ends. And the serum of patients with limb fracture combined with brain injury can significantly promoting the mitosis of osteoblast cells and proliferation of the bone marrow stem cell[7]. It can also affect the fluid circulation mediated by brain-derived factor, thus further promote the healing of fractures and the expression of the correlation factors in the serum.

NGF is a polypeptide hormone containing 118 amino acids, and it plays an important role in survival of nerve center and peripheral neurons. It can adjust their growth, development and differentiation process[8]. The serum NGF levels gradually increased in limb fracture combined with brain injury group, traumatic brain injury group and simple fracture group. It suggested that a large number of growth factors will secreted in the body of the patient´s after injury. NGF synthesis and release increase in early stage of brain injury and bone injury. The serum of NGF levels at 1 d, 3 d, 7 d, 14 d after injury in limb fracture combined with brain injury group were significantly higher than those of the simple fracture group and the traumatic brain injury group (P<0.05). The serum of NGF levels at 1 d, 3 d, 7 d, 14 d after injury in the traumatic brain injury group were significantly higher than the simple fracture group (P<0.05). It suggested that secretion of NGF in patients with limb fracture combined with brain injury showed a significant upward trend, similar to the research results of Yang[9]. In this study, we presume that the secretion of NGF can affect the healing of fracture after the trauma of central nervous system. The possible mechanism of that is the NGF can combine the osteogenic cell membrane receptors, which enhanced the activity of the bone cells in the process of the phosphorylation of the osteoblasts, and enhanced the osteogenic capacity and promoted fracture healing. NGF can induced the nerve ingrowth the callus, and can increase the local blood supply, so as to promote the bone regeneration.

EGF is a polypeptide growth factor formed by 53 amino acids which is widely distributed in the body. It is a powerful broad spectrum cell division promoter[10], and can simultaneously act on vascular endothelial cells, fibroblasts and osteoblasts. EGF receptor exists in all organizations of the human body except the hematopoietic system[11]. Kong[12] confirmed the serum EGF concentrations of the fracture rabbit increased significantly through establishing a fracture model in rabbit, and the concentration differences has statistically significant compared with the normal group. The serum EGF concentrations of the traumatic brain injury group and the simple fracture group show a slow upward trend. The serum levels of EGF at 1 d, 3 d, 7 d 14 d after injury in limb fracture combined with brain injury group were significantly higher than those of the simple fracture group and the traumatic brain injury group (P<0.05). The serum levels of EGF at 1 d, 3 d, 7 d 14 d after injury of the traumatic brain injury group were significantly higher than the simple fracture group (P<0.05). That suggested the EGF concentrations of the limb fracture combined with brain injury group increased significantly, and reached its peak after one week of the injury. This study confirmed that EGF is closely related to the cells growth, proliferation, differentiation, and regeneration of the central nervous system. EGF can promote the formation of bone tissue, and accelerate the synthesis and deposition of the matrix. Early supplement of exogenous NGF and EGF can promote the recovery of the central nervous system damage, it can also accelerate the healing of bone fractures, which provide a new method for the clinical treatment of patients with limb fracture combined with brain injury.

Conflict of interest statement

We declare that we have no conflict of interest.

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