European Review for Medical and Pharmacological Sciences 2017; 21: 861-866

Correlation analysis of mast cells and EGFR with endoscopic application of tissue glue for treatment of peptic ulcer healing

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Abstract. – OBJECTIVE: To investigate the impact of tissue glue on mast cells (MC) and epidermal growth factor receptor (EGFR) in rat peptic ulcer.

MATERIALS AND METHODS: SD rats were used to establish peptic ulcer model. Cimetidine gavage was adopted in the positive control, while cimetidine and endoscopic tissue glue therapy were applied in the experimental group. The ulcer inhibition rate and ulcer index were measured to evaluate healing quality. Real-time PCR was performed to test EGFR mRNA in the ulcer surrounded gastric mucosa. Immunohistochemistry was selected to determine MC quantity. HE staining and apoptosis detection were used to evaluate cell apoptosis.

RESULTS: Tissue glue significantly reduced MC number in the peptic ulcer rat compared with control with dose dependence ($p < 0.05$). Tissue glue significantly decreased ulcer area, and elevated ulcer index and inhibition rate ($p < 0.05$). EGFR mRNA in the mucosa markedly declined after modeling. Tissue glue upregulated EGFR mRNA to a certain extent ($p > 0.05$). Tissue glue induced MC apoptosis with dose dependence.

CONCLUSIONS: Endoscopic application of tissue glue accelerated ulcer mucosa healing via up-regulating EGFR mRNA, enhancing gastrointestinal mucous membrane regeneration and repair ability, and decreasing MC number.

Key Words:
Peptic ulcer, Endoscopic tissue glue, Healing quality, Mast cell, EGFR.

Introduction

Peptic ulcer, a common disease in digestive system, refers to the mucosal ulcer caused by pepsase and gastric acid auto-digestion. It exhibits complex etiology and long-term recurrent attack[1]. The etiology and pathogenesis of peptic ulcer are still unclear. At present, the imbalance between damage factor and defending factor is the main cause of ulcer[2,3]. Damage factors include drugs, pepsase, gastric acid, prostaglandin, epidermal growth factor, *Helicobacter pylori*, spirit, and genetic factors. The healing of peptic ulcer is a conundrum of its treatment. Various histological and ultra-structural abnormalities affect the defense of mucous membrane and cell oxygenation capacity, becoming the physiological and pathological bases of ulcer healing and recurrence. Its treatment not only needs to repair mucosal deletion, but also rebuild the sub-mucosal tissue[4]. The mucosal healing process is affected by growth factor and its receptor, leading to stimulate the digestive tract mucosa cells proliferation, exert the regulatory function of the growth factors, promote the gastric mucosal blood flow, inhibit gastric acid secretion, facilitate mucosal healing, and exhibit strong mucosal cell protection. Current studies found that epidermal growth factor receptor (EGFR) showed good digestive tract mucosal protection. Its expression in normal gastric mucosa is few, while gastrointestinal damage can increase its level. It can stimulate epithelial cells and surrounding cells proliferation, with high affinity of the digestive tract mucosa and healing effect to the ulcer surface[5]. Also, there are various mast cells (MC) with multiple biological activities and different anatomical parts that have different impact on inflammation. In the digestive tract, MC release mediator to promote muscle contraction, increase vascular permeability and mucous secretion, and accelerate the inflammatory cells stimulating the pain nerve, cause the digestive

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tract local edema, weaken uptake of nutrients, give rise to local blood loss, and generate pain[6]. It is considered that MC involved in the occurrence, development, and healing of peptic ulcer[7]. In recent years, endoscopic application of tissue glue obtained good clinical effect, with the role of hemostasis and promoting local inflammation resolution. However, its treatment mechanism is still controversy. Therefore, we explored the role of endoscopic tissue glue on peptic ulcer healing and related mechanism through using the animal model, aiming to provide new thought for the treatment and prevention of peptic ulcer.

Materials and Methods

Experimental Animal

Male SD rats weighted 180-220 g were bought from Beijing Animal Center (license key: SCXK 2016-0263). The rats were raised in SPF grade animal feeding room with temperature at 21-24°C, humidity at 40%-60%, free eating and drinking, and 12 h day-night cycle.

Rats were used for all experiments, and all procedures were approved by the Animal Ethics Committee of Beijing Armed Police General Hospital, Beijing, China.

Drugs and Reagents

EGFR and MC were got from Sigma-Aldrich (St. Louis, MO, USA). Endoscopic tissue glue was obtained from Ankang Chia Tai Pharmaceutical co., Ltd (Beijing, China). Biochemistry kit was purchased from Merlin medical science co., Ltd (Shanghai, China). PCR primers were constructed by Sangon (Shanghai, China).

Instruments

Injection needle, GIF-Q150 electronic gastroscope, histotome, Real-time PCR amplifier, microplate reader, high-speed centrifuge, embedding machine, and analytical balance were purchased from Olympus (Tokio, Japan). α-butylcyanoacrylate tissue glue at 0.5 ml was obtained from Suncon Medical Adhesive (Beijing, China).

Experimental Method

Animal Model Establishment

A total of 80 male SD rats were randomly divided into two groups, including 70 in the experimental group and 10 in the negative control. The rats in experimental group were anesthetized by 1% pentobarbital sodium (Haoran Bio. Ltd., Beijing, China) and given 0.002 ml 100% glacial acetic acid (Haoran Bio. Ltd., Beijing, China) injection at the junction of the antrum of the stomach and gastric body to construct the peptic ulcer animal model. When the white ulcer ring on the serosal layer reached 3 mm, the stomach was returned to the abdominal cavity. Tissue glue was endoscopically injected at 1.5 ml, 1 ml, or 0.5 ml for 2 days, respectively. The endoscopic normal saline injection was selected as normal control. The rats in sham group were opened abdominal cavity without injection.

Index Observation

After two days' treatment, the rat was anesthetized to extract blood from the abdominal aorta. The serum was separated, while the gastric mucosa was put into the liquid nitrogen and embedded after paraformaldehyde (Sigma-Aldrich, St. Louis, MO, USA) fixation.

Mast Cells HE Staining and Apoptosis Detection

Apoptosis index (AI) = apoptotic cell number/total cell number × 100%. Apoptotic MC was stained by TUNEL kit (Roche, Mannheim, Germany) and observed under the microscope (Olympus, Tokyo, Japan). The apoptotic cytoplasm was stained as yellow, whereas the cell nucleus was stained as hyacinthine. The apoptotic cells were accounted to calculate AI.

EGFR mRNA Detection

PCR reaction was performed at pre-degeneration at 95°C for 2 min, followed by 40 cycles of 95°C for 20 s, 62°C for 30 s, and 68°C for 60 s. Total RNA was extracted from gastric mucosal tissue using Trizol method. Real-time PCR was used to test EGFR mRNA expression. The primers sequences were as follows. EGFR mRNA, forward, 5'-ACTCGCAGGAAAGACTAGCA-3', reverse, 5'-AGCAGTGGAAGAATCGGACC-3'. GAPDH, forward, 5'-CATCAGCAATGCCTCCTGCAC-3', reverse, 5'-TGAGTCCTTCCACGATACCAAAGTT -3'.

Statistical Analysis

All data analyses were performed by SPSS 20.0 software (SPSS, Inc., Chicago, IL, USA). Enumeration data were compared by x^2-test, while measurement data were analyzed by t-test. $p < 0.05$ was depicted as statistical significance.

Table I. The impact of tissue glue on MC number in rat serum.

Group	n	Un-degranulation	Degranulation (%)
Control	10	10.05 ± 4.39	15.84
Model	10	26.46 ± 7.60**	39.34**
High dose tissue glue	10	13.21 ± 3.41##	24.23##
Middle dose tissue glue	10	20.72 ± 11.03#	28.5#
Low dose tissue glue	10	22.22 ± 8.51#	29.64#
Positive control	10	16.50 ± 8.11##	28.6##

*$p < 0.05$, **$p < 0.01$, compared with control. #$p < 0.05$, ##$p < 0.01$, compared with model group.

Results

The Impact of Tissue Glue on MC Number in Rat Serum

Tissue glue significantly reduced MC number in the peptic ulcer rat compared with control with dose dependence ($p < 0.05$) (Table I).

The influence of Tissue Glue on Ulcer Area, Ulcer Index, and Ulcer Inhibition Rate

Tissue glue obviously decreased ulcer area, and elevated ulcer index and inhibition rate with dose dependence ($p < 0$ .05) (Table II).

The Effect of Tissue Glue on EGFR mRNA Expression in Ulcer Surrounding Gastric Mucosa

EGFR mRNA in the mucosa markedly declined after modeling. Tissue glue up-regulated EGFR mRNA to a certain extent without statistical significance ($p > 0.05$) (Figure 1).

The Impact of Tissue Glue on Gastric Mucosa Pathologic Histology

HE staining revealed that the cells distributed in crumby structure, whit normal cell number, abundant capillary, and without degeneration or necrosis (Figure 2A). B cells number reduced in model group, accompanied by necrosis, compensatory swelling, and angiotelectasis (Figure 2 B). They were improved in high, middle, and low dose group compared with model group, including B cells deletion in number, compensatory swelling, and B cells necrosis (Figure 2 C-E). Part of cells appeared compensatory swelling, B cell apoptosis, and acinus cavity (Figure 2 F).

The Influence of Tissue Glue on MC Apoptosis

It was showed that no MC apoptosis was observed in normal control (Figure 3 A). The model group exhibited karyopyknosis and DAB yellow staining, indicating MC apoptosis (Figure 3 B). Tissue glue improved MC apoptosis compared with the model group with dose dependence (Figure 3 C-E).

Discussion

Peptic ulcer is a clinical common disease, featured as mucosal defense and repair ability reduction, and regeneration epithelial tissue damage[8]. The previous study reported that the quality of primary ulcer healing is of great importance for the disease. However, abnormal structure reduces the energy supply and cell oxidation, which is the pathological basis of ulcer recurrence. Therefore, assessing the quality of ulcer healing needs

Table II. The influence of tissue glue on ulcer area, ulcer index, and ulcer inhibition rate.

Group	n	Ulcer area (cm^2)	Ulcer index	Ulcer inhibition rate (%)
Control	10	8.02 ± 3.24	0.69 ± 0.21	–
Model	10	11.31 ± 4.33**	9.33 ± 0.50*	–
High dose tissue glue	10	12.35 ± 3.37#	1.04 ± 0.45#	87.43#
Middle dose tissue glue	10	14.13 ± 1.96	4.46 ± 0.20#	56.58
Low dose tissue glue	10	14.93 ± 3.07	4.49 ± 0.39#	51.42
Positive control	10	14.97 ± 2.52	1.33 ± 0.24	79.37

*$p < 0.05$, **$p < 0.01$, compared with control. #$p < 0.05$, ##$p < 0.01$, compared with model group.

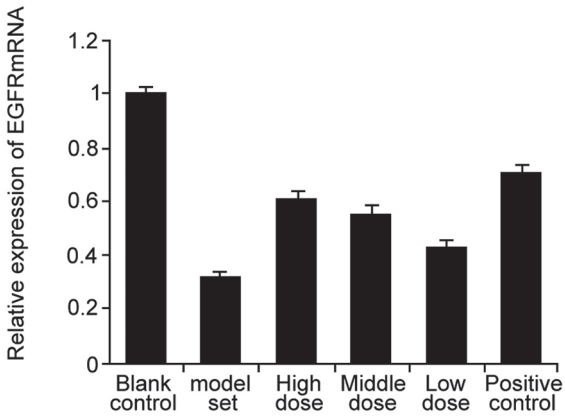

Figure 1. Relative EGFR mRNA expression in gastric mucosa surrounding the ulcer.

to evaluate the maturity of regenerative mucosa and attach importance of the function maturity[9-11]. Different quality of ulcer healing is closely related to recurrence. Thus, improving the quality of ulcer healing is the key to the prevention of recurrent ulcer[12,13].

At present, the main treatment method in clinic includes endoscopic mucosal repair, inhibition of gastric acid, protecting mucous membrane, and the eradication of *Helicobacter pylori*. Traditional drug treatment can obtain a relatively good short-term curative effect, but it is difficult to control relapse[14]. Endoscopic treatment exhibits the advantage of small trauma and better effect of local medication[15]. In recent years, our department adopted tissue glue surrounding mucosa injection for the ulcer treatment, effectively preventing ulcer recurrence, promoting local digestive tract mucosal repair, and improving the quality of ulcer healing. Tissue glue is not absorbed by the tissue, and also can be discharged through the blood vessel. It can rapidly block the blood vessel, leading to local tissue congestion. Moreover, it may occur polymerization reaction with touched blood in seconds to form adhesive solid appearance, which is completely separated from the blood vessels and blocks blood vessels without causing local vasculitis and vascular fibrosis.

EGFR concentrates in the cytoplasm of mucus neck cells, parietal cells, gastric mucosa in the regenerative zone, and cell membrane. It is low expressed in normal gastric mucosa epithelial cells, lamina propria cells, and mucosal myocytes[16].

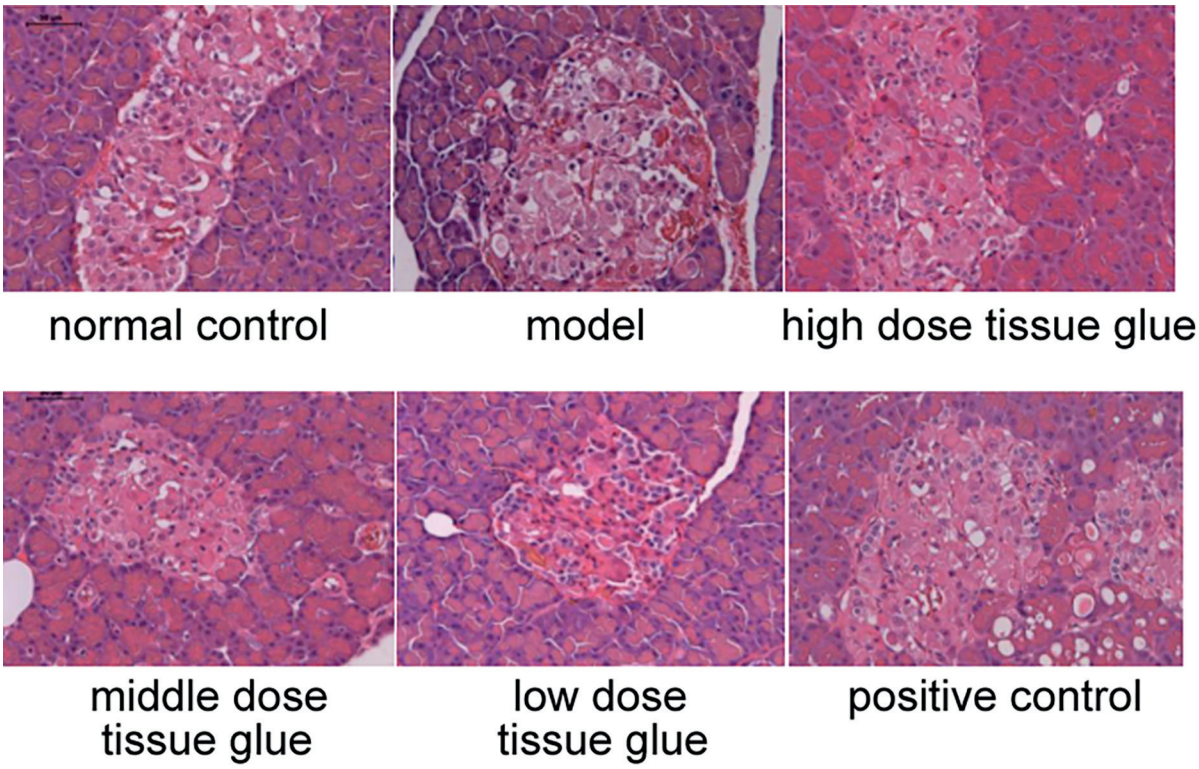

Figure 2. The impact of tissue glue on gastric mucosa pathologic histology (×400).

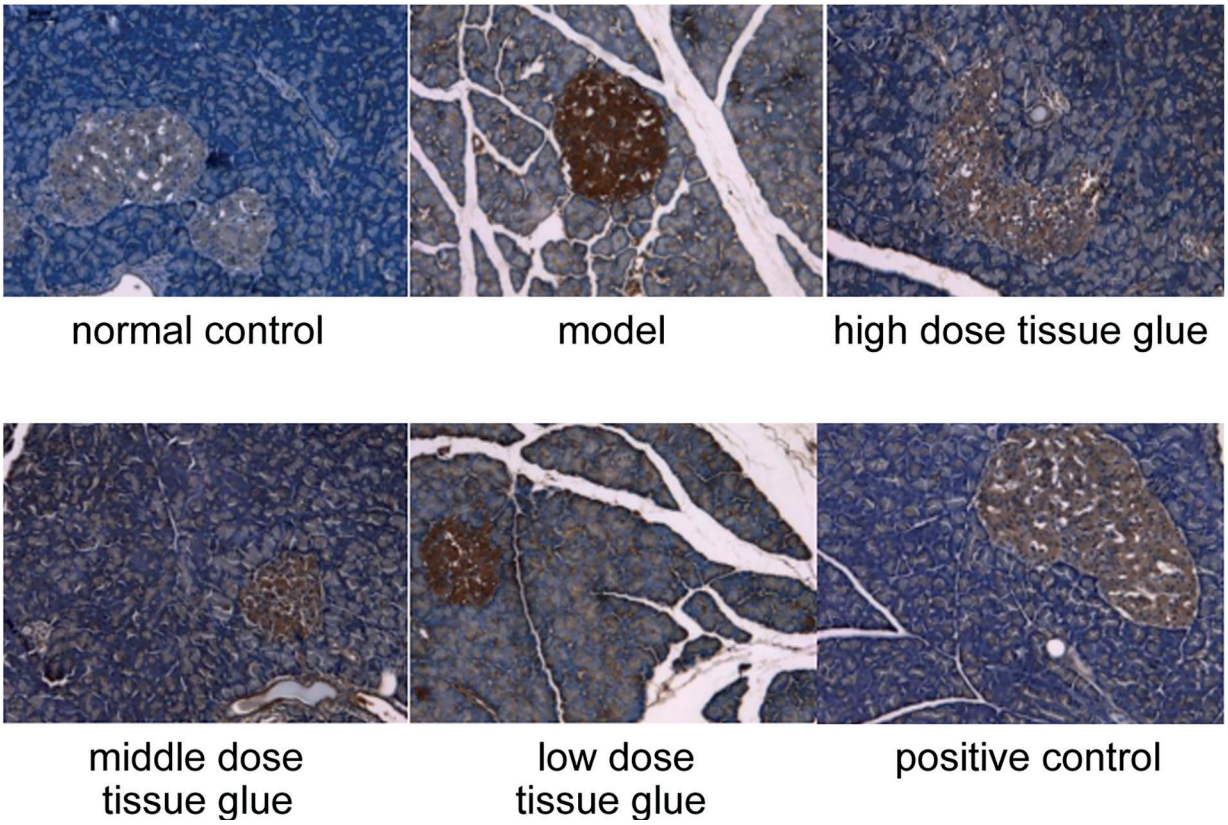

Figure 3. The influence of tissue glue on MC apoptosis (×400).

EGFR significantly increases during gastric mucosa damage. It mainly distributes in epithelial cells. Gastric mucosa cells are the target of EGF, which can improve the quality of ulcer healing, and promote ulcer healing and tissue repair via binding with EGFR. Currently, EGFR is considered as an important evaluation indicator of the quality of ulcer healing[17].

MC is derived from hematopoietic progenitor cells. Its surface shows high affinity with IgE immune adjustment, resulting in the opening of biological active media channel. MC contacted with outside antigen can generate good stimulative effects on the microenvironment. However, the protease is different between mucosal connective tissue and MC[18]. Current research proposed that MC participates in the process of digestive tract ulcer. It was also found that MC quantity and degranulation increased under ulcer and mucosal damage. Degranulation rate was markedly higher than normal tissue[19]. Histamine releases after MC degranulation, therefore, promoting the local release of bioactive substance, angiectasis, increasing the permeability of blood vessel, and causing the stomach function obstacle. It further stimulates MC degranulation, promotes the diffusion of hydrogen ion to the gastric mucosa, thus involving in gastric ulcer formation[20-22]. This study investigated the correlation relationship between EGFR and MC with the quality of peptic ulcer healing.

Conclusions

Our results revealed that tissue glue significantly reduced MC number in the peptic ulcer rat compared with control and dose dependence. Compared with model group, tissue glue obviously decreased ulcer area, and elevated ulcer index and inhibition rate. EGFR mRNA in the mucosa markedly declined after modeling. Tissue glue up-regulated the EGFR mRNA to a certain extent. MC participates in the amplification reactions of inflammation. More MC and more serious degranulation lead to more severe of the mucous membrane inflammation,

leading to body damage. After treatment, tissue glue induced MC apoptosis with dose dependence. Therefore, tissue glue effectively inhibits inflammation, suppresses EGFR reaction to inflammatory cytokines, and reduces MC generation, thus is advantageous to the quality of ulcer healing.

Acknowledgements
This work was supported by Capital Medical Development and scientific research fund (NO. Z151100004015213).

Conflict of Interest
The Authors declare that they have no conflict of interests.

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