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Protective effect of polaprezinc on acute gastric mucosal injury in rats

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ABSTRACT

Objective: To explore the role of prostaglandin E2 (PGE2) and epidermal growth factor (EGF) in the protective effect of polaprezinc on acute gastric mucosal damage.

Methods: A total of 30 male SD rats were randomly divided into 3 groups as follows: A blank group, a model group, and a polaprezinc group. The blank group was fed normally. As for the model group and the polaprezinc group, the rats were given 1 mL ethanol (intragastrical administration) first, then they were treated with vehicle (1 mL distilled water) or polaprezinc treatment (100 mg/kg) 1 h later. On the 3rd day, rats in each group were fasting for 24 h before gastric administration. After 2 h of gastric administration, 5 mL of intraperitoneal blood was collected, centrifuged and stored at –80 ℃ for the detection of EGF and PGE2. Gastric tissue was collected for anatomic and pathological assessment.

Results: Polaprezinc reduced gastric mucosa injury in the polaprezinc group compared to the model group. Compared with the blank group, the levels of PGE2 and EGF in blood serum were significantly decreased in the model group and the polaprezinc group (P<0.05), while there was no significant difference between the model group and the polaprezinc group (P>0.05).

Conclusion: Polaprezinc can provide effective protection for acute mucosal injury and the underlying mechanism is not directly related to PGE2 and EGF.

KEY WORDS polaprezinc; acute erosive gastritis; prostaglandin E2; epidermal growth factor

聚普瑞锌对大鼠急性胃黏膜损伤的修复作用

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[摘要] **目的：**探讨聚普瑞锌促进大鼠酒精性急性胃黏膜损伤的修复作用机制及与前列腺素E2(prostaglandin E2，

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PGE2)、表皮生长因子(epidermal growth factor，EGF)的关系。**方法：**30只SD大鼠随机分为空白组、模型组、治疗组，每组10只。空白组给予常规饲养，其余两组给予无水乙醇1 mL制造急性糜烂性胃炎模型。1 h后治疗组予1 mL聚普瑞锌溶液(按聚普瑞锌100 mg/kg体重计算)，并连续予聚普瑞锌灌胃3 d，每天1次；模型组则给予1 mL蒸馏水灌胃，连续3 d，每天1次。第3天灌胃前各组大鼠均禁食24 h，灌胃2 h后腹腔取血5 mL，离心取血清置EP管中，–80 ℃冰箱保存待测EGF和PGE2水平，并取大鼠胃行大体形态及病理组织观察。**结果：**治疗组大鼠胃黏膜大体及病理组织观察均较模型组愈合好。与空白组比较，模型组、治疗组血清中PGE2和EGF含量明显降低，差异有统计学意义(P<0.05)，治疗组与模型组PGE2和EGF含量比较，差异无统计学意义(P>0.05)。**结论：**聚普瑞锌能促进急性胃黏膜损伤的修复，但其作用机制不受PGE2和EGF的调节。

[关键词] 聚普瑞锌；急性糜烂性胃炎；前列腺素E2；表皮生长因子

Acute gastric mucosal injury is one of the common causes of upper gastrointestinal bleeding (accounts for 20%–30%) and its mechanism is related to the imbalance of attack factor and defensive factor. Zinc-left-circumflex muscle peptide (polaprezinc) is a chelating compound composed of zinc ions and *L*-type muscle peptides, a new type of anti ulcer drug with protective effect of gastric damage induced by immersion stress, ischemia-reperfusion, aspirin, ethanol or histamine in many experimental models[1-3]. Zinc is a component of enzymes and transcription factors, which have several biological functions, such as participating in nucleotide synthesis, protein synthesis, and gene expression during cell proliferation and differentiation. Zinc deficiency can delay ulcer healing and zinc is important for wound healing[4]. *L*-peptide is composed of two amino acids, *L*-histidine and β-alanine, which are usually found in skeletal muscle and can effectively promote wound healing, including the healing of gastric ulcer[5]. A large number of in vitro and in vivo studies[6-8] have confirmed that prostaglandin E2 (PGE2) can inhibit the secretion of gastric acid and pepsin, stimulate the secretion of mucus and bicarbonate, increase mucosal blood flow and epithelial regeneration, and enhance mucosal resistance (cell protection). Epidermal growth factor (EGF) is a molecule that plays an important role in cell proliferation and differentiation in recent years. It is an endogenous substance that inhibits gastric acid secretion, promotes epithelial cell proliferation, tissue repair and cell protection. It plays an important role in protecting gastric mucosa from damage factors and maintaining the integrity of gastrointestinal mucosa. It has been proved that PGE2 and EGF have protective effects on the gastric mucosa, but whether it is regulated by PGE2 and EGF in the process of accelerating gastric mucosal is still unknown. In this study, we established a rat model of acute gastric mucosal injury with ethanol, by comparing the level of PGE2 and EGF in blood serum model to explore their role in polaprezinc induced mucosal repair.

1 Materials and methods

1.1 Animals

A total of 30 male SD rats (weight 250–300 g) were purchased from the Animal Experiment Center of Central South University, provided with the standard laboratory free diet and water, and kept in a controlled cage at a temperature of (22±2) ℃ and humidity (55±5)%. These 30 SD rats randomly divided into 3 groups: A blank group, a model group, and a polaprezinc group, each group of 10.

The rats were kept in the same environment for 1 week before experiment.

1.2 Reagents and instruments

Polaprezinc was purchased from Jilin Boda Weiye Pharmaceutical Co., Ltd. Jilin Great Albert); EGF reagent box and PGE2 kit were purchased from Wuhan Huamei Bioengineering Co., Ltd.); anhydrous ethanol, saline, distilled water, refrigeration centrifuge, automatic enzyme labeling machine washer, enzyme labeling instrument, constant temperature oscillator, and other common instruments were purchased from Central South University Departmenf of Laboratory Animals.

1.3 Methods

1.3.1 Animal model construction and serum collection

The blank group was fed normally. As for the model group and the polaprezinc group, the rats were given 1 mL ethanol (intragastrical administration) first, then they were treated with vehicle (1 mL distilled water) or polaprezinc treatment (100 mg/kg) 1 h later. On the 3rd day, rats in each group were fasting for 24 h before gastric administration. After 2 h of gastric administration, 5 mL of

intraperitoneal blood was collected, centrifuged and stored at –80 ℃ for the detection of EGF and PGE2.

1.3.2 Gross and pathological examination of gastric mucosa

The gastric tissue of rats was dissected and separated after the abdominal aorta was taken blood, the blood and stomach contents of gastric mucosa with 0.9% sodium chloride saline solution were flushed, the general situation of gastric tissue mucosa were observed, gastric mucosa lesions were selected, removed, and fixed 24 h in 10% formaldehyde solution, then observed by HE staining.

1.3.3 Detecting PGE2 and EGF in blood serum

Enzyme-linked immunosorbent assay (ELISA) was used to step sandwich with double antibody. In the package of E2 (PGE2) antibody to the prepackaged prostate gland, the test antibody of the specimen, the standard product and the HRP mark were added in sequence after the warm-bred and thoroughly washed. The 3,3',5,5'-tetramethylbenzidine (TMB) was converted to blue by the TMB of the substrate and converted to the final yellow under the action of the acid. The depth of the color was positively correlated with the level of PGE2 in the sample. The absorbance was measured at 450 nm wavelength and the sample concentration was calculated. The level of EGF in serum was detected by the same method as mentioned above.

1.4 Statistical analysis

SPSS 19.0 statistical software was used for data analysis. All data in the text and figures were expressed as mean±standard deviation ($\bar{x}\pm s$). Two or more group comparisons were evaluated by one-way analysis of variance (ANOVA). Differences were considered statistically significant at $P<0.05$.

2 Results

2.1 General situation

In the blank group, rats were in good condition, with normal feeding, bright hair, and good mental state. In the model group and the polaprezinc group, rats' spirit significantly worse and appetite decreased to quarter after modeling. The spirit and appetite of the rats were improved obviously after the treatment, and the improvement of symptoms in the treatment group was more obvious than that in the model group.

2.2 Specimens of gastric mucosa in the 3 groups

The gastric mucosa in the blank group was pink, glossy, covered with more mucus, smooth surface of mucous membrane, shape rules, and good elasticity of gastric wall. In the model group, the gastric wall elasticity was weakened, the mucosa was thin, the color was pale, the folds were flat or shape, and the mucosa of some rats' antrum was different in point shape or lesion. The naked eye observation of the gastric mucosa in the polaprezinc group was better than that in the model group and the blank group (Figure 1).

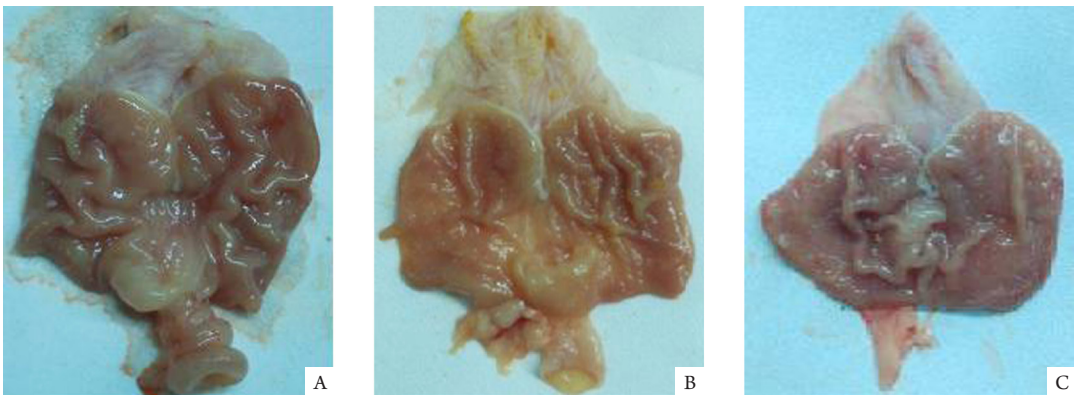

Figure 1 Specimens of gastric mucosa in the 3 groups

A: Blank group; B: Model group; C: Polaprezinc group

2.3 Pathological phenomena of gastric mucosa under optical microscope

In the blank group, the surface of gastric mucosa was not damaged or exfoliated, and inflammatory cells were rare. The gastric mucosa of the model group was damaged, exfoliated and infiltrated with a large number of inflammatory cells under the mucosa. Compared with the model group, the gastric mucosa of the treatment group treated with 1 mL of polyprezine (100 mg/kg) for 3 days was less damaged and slightly exfoliated, and the inflammatory cell infiltration was less, similar to that of the control group (Figure 2).

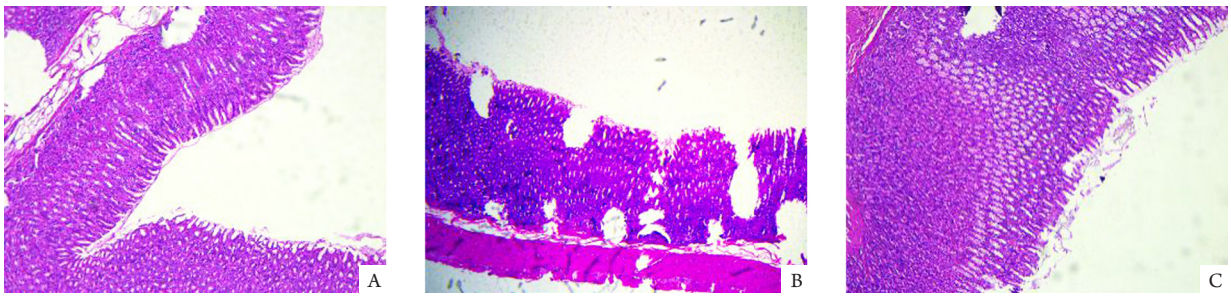

Figure 2 HE staining in the 3 groups (×100)

A: Blank group; B: Model group; C: Polaprezinc group

2.4 Levels of PGE2 and EGF in blood serum

Compared with the blank group, the levels of PGE2 and EGF in blood serum were significantly decreased in the model group and the polaprezinc group (P<0.05), while there was no significant difference between the model group and the polaprezinc group (P>0.05, Table 1).

Table 1 Comparison of serum PGE2 and EGF levels in the 3 groups ($\bar{x}\pm s$)

Groups	PGE2/(ng·mL^{-1})	EGF/(ng·mL^{-1})
Blank group	35.61±9.84	23.69±2.56
Model group	5.56±1.53*	11.24±2.25*
Polaprezinc group	5.02±1.98*	9.67±1.27*

*P<0.05 vs the blank group

3 Discussion

Polaprezinc can adhere to the ulcer site and longer than zinc or *L*-peptide in the stomach. This suggests that the insoluble property of polaprezinc has a stronger pharmacological effect[9]. Although the detailed mechanism for promoting ulcer healing is unknown, the function of polaprezinc can be explained by stimulating mucus production , antioxidant effects[10], and membrane stability[11]. Polaprezinc can preserve the barrier of gastric mucosa through its stabilizing effect and exert protective effect on gastric lesions caused by ethanol[12]. Zinc ions and carnosine are both responsible for the antioxidant properties of poly. In addition, in a certain degree, the presence of *L*-actin alone cannot inhibit the gastric mucosal lesions caused by ethanol. The mechanism may partly be explained by zinc ions and *L*-peptide may enhance the protective effect of zinc ions. Although the detailed mechanism for ulcer healing is not clear, some studies[13-15] showed that polaprezinc can play an important role in protecting gastric mucosal lesions by inducing heat shock protein (HSP) formation. It also played a role in inducing insulin-like growth factor-1 (IGF-1) and superoxide dismutase (SOD) production and repairing gastric mucosal lesions.

Prostaglandin (PGs) is a kind of important mucosal protective factor. Many kinds of drugs or physicochemical factors can regulate the synthesis of PGs, and then affect the repair of gastric mucosa injury. PGE2 plays an important role in protecting gastric mucosal barrier and participating in the regeneration and repair of epithelial cells. It is considered that the mechanism of PGs for the

repair of peptic ulcer might be: protecting the gastric mucosal barrier, promoting gastric mucosal cell renewal, stimulating the cell's active transport process and activating the adenosine propionate enzyme, improving the gastric mucosal blood flow, stabilizing the lysozyme and maintaining the content of the radical compounds in the gastric mucosa, increasing the content of phospholipid in the gastric mucosa, and inhibiting the main cells to reduce the secretion of gastric proteas[16].

EGF is a single chain polypeptide hormone containing 53 amino acid residues, which promotes the synthesis of DNA, RNA and protein, promotes mucosal epithelial proliferation and promotes gastric ulcer healing. Some studies[17-18] have confirmed that the level of EGF in gastric ulcer patients is significantly lower than that of normal people, suggesting that the decrease of EGF may be related to the occurrence of gastric ulcer. The content of EGF in gastric juice increased significantly after ulcer healing, suggesting that EGF may play a role in ulcer healing. The mechanism of EGF on the repair of peptic ulcer may be: EGF has nutrition effect on gastrointestinal mucosa, maintains the integrity of mucosal barrier in gastrointestinal tract, suppresses gastric acid secretion, promotes the synthesis and secretion of mucous glycoprotein in gastric mucosa, promotes the repair of damaged mucosa, improves the microcirculation of gastric mucosa, strengthens the adaptability of gastric mucosa, To stimulate the migration and mitosis of gastric epithelial cells, the protective effect of EGF on gastric mucosa may be mediated by PGs, but the mechanism of its specific action remains to be further studied.

At present, the study on the mechanism of promoting the gastric mucosa healing by polaprezinc is rare. The pathogenesis of acute gastric mucosal lesions is mainly caused by the imbalance of gastric mucosal defenses and attack factors, including mucus-carbonate barrier, mucosal barrier, mucosal blood flow cell renewal, PGs, EGF, and so on. This research studied the effect of polaprezinc on the healing of acute gastric mucosal lesions induced by ethanol in rats and its relationship between PGE2 and EGF. Compared with blank group, the levels of PGE2 and EGF in blood serum were significantly decreased in the model group and the polaprezinc group (P<0.05), while there was no significant difference between the model group and polaprezinc group (P>0.05). It is concluded that the decrease of PGE2 is one of the causes of acute gastric mucosal injury induced by ethanol. The healing mechanism of polaprezinc in acute gastric mucosal injury is not related to PGE2. Arakawa et al[19] studied the effects of zinc on alcohol-induced gastric mucosa and cell damage in rats and the relationship between endogenous PGE2 and gastric mucosal protection. The results showed that zinc *L*-carnosine could protect gastric mucosa and enhance cell tolerance to alcohol. However, the protective effect of zinc *L*-carnosine in vivo seems not to be regulated by PGs, because zinc *L*-carnosine can not affect the content of PGs in gastric mucosa, and indomethacin can not inhibit the mucosal protection caused by zinc *L*-carnosine. The content of PGE2 in rat serum was determined in this experiment, and the results were consistent with the above results. The mechanism of zinc *L*-carnosine against the mucosal protective effect of alcohol is not clear, it may be related to zinc compound stimulating mucous secretion of gastric mucosa and zinc inhibiting the instability of lysozyme cell membrane. Specific mechanisms need to be further studied. Compared with the blank group, the serum EGF levels in the model group and the polaprezinc group were significantly lower, but there was no significant difference between the model group and the polaprezinc group, which indicated that the acute gastric mucosal injury induced by ethanol could lead to the decrease of serum EGF levels in rats. However, the mechanism for promoting gastric mucosal healing by polaprezinc may not be regulated by EGF. The mechanism of EGF may be mediated by PGs. Some international studies have proved that PGs can not regulate the promotion of gastric mucosa healing by polaprezinc, our results also confirmed that PGE2 does not increase in the promotion of gastric mucosa healing with poly-zinc. Therefore, EGF can not play a role in polaprezinc promoting gastric mucosa healing by PGE2.

By this experiment, polaprezinc can provide effective protection for acute mucosal injury and the underlying mechanism is not directly related to PGE2 and EGF. The specific mechanism of promoting the healing of the mucosa needs further study.

Conflict of interest: The authors declare that they have no conflicts of interest to disclose.

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