Protection of gastric mucosa from ethanol induced injury by recombinant epidermal growth factor in rats

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Abstract
AIM To determine whether recombinant human epidermal growth factor (rhEGF) can protect gastric mucosa against ethanol induced injury in rats.
METHOD Fifty-four SD rats weighing 200g - 500g each were divided into six groups after fasting for 24 hours. Three groups received different doses of oral rhEGF (30, 60 and 120µg·kg⁻¹·d⁻¹), one group was given cimetidine, one subcutaneous rhEGF (rhEGF IV) and one received saline as control.
RESULTS Acute gastric dilatation developed in the control and cimetidine groups and bloody gastric juice was found in the control group. The ulcer index was 58 in control group, 53 in rhEGF I, 46 in rhEGF II (P<0.01), 11 in rhEGF III (P<0.01), 19 in rhEGF IV (P<0.01), and 39 in cimetidine group (P<0.05).
CONCLUSION rhEGF protected gastric mucosa against ethanol induced damage. The effect was dose-dependent with blood levels of epidermal growth factor (EGF) at a dosage range of 60µg·kg⁻¹·d⁻¹ - 20 µg· kg⁻¹·d⁻¹. It was more effective by injection than via oral route at the same dosage.

INTRODUCTION
Epidermal growth factor (EGF) is a single-chain polypeptide that is secreted by submandibular and Brunner’s glands and is a powerful mitogen and an inhibitor of gastric acid secretion. Recent studies demonstrated that EGF is also capable of protecting the gastric mucosa from the damage caused by various irritants and promoting healing of chronic gastric and duodenal ulcers[1,2]. This study was designed to determine whether recombinant human EGF (rhEGF) could protect gastric mucosa against ethanol induced injury in rats. Our aim is to find a new method of gastric mucosa protection which may serve as a treatment for peptic ulcer.

MATERIAL AND METHODS
SD rats weighing 200 to 250g were used in the study of gastric protection and gastric ulcer.
Experiments with acute gastric mucosal lesion. Acute gastric lesion was induced by absolute ethanol in experiments with three sets of rats (control, rhEGF and cimetidine). The control group was given 0.9% saline for 3 days and then 100% ethanol (9 rats). Cimetidine group was treated with cimetidine and then with 100% ethanol (9 rats). The rhEGF group was divided into 4 subgroups, each received oral rhEGF 30, 60 or 120 µg·kg⁻¹·d⁻¹ except one subgroup which was given 60µg·kg⁻¹·d⁻¹ rhEGF subcutaneously. Three days later 1mL of absolute ethanol was administered to all rats. One hour after ethanol administration, the rats were killed by cervical fracture. The stomach was dissected out and opened along the greater curvature, and the area of ulceration was determined. All planimetric determinations were performed blindly by the same observer.
The measurement of serum EGF and gastrin level. Rat blood of 2 ml - 4 ml was collected in tube without anticoagulant. 3 hours later the serum was collected and EGF and gastrin measured. EGF kit was obtained from Amersham, U.K. and Depu Co, China.

RESULTS
Gastric ulcer index
Two rats in cimetidine group died in less than 1 hour. After administration of ethanol, acute gastric dilatation developed in the control and cimetidine groups and bloody gastric juice was found in the control group. The ulcer index was 58 in the control group, 53 in rhEGF I, 46 in rhEGF II (P<0.01), 11
in rhEGF III (P<0.01), 39 in rhEGF IV (P<0.01), and 39 in cimetidine group (P<0.05, Table 1).

The levels of serum EGF. The levels of serum EGF in rhEGF III, IV and cimetidine groups were higher than that of control group (P<0.05), especially in rhEGF III group (P<0.001). After giving the same dose of rhEGF, the serum EGF level in the subcutaneous group was significantly higher than that of the oral groups (Table 2).

The values of serum gastrin. The values of serum gastrin in rhEGF IV group was increased significantly than the control group (P<0.05, Table 3).

| Table 1 | Index of gastric ulcer in all groups (M) |
|---------|----------------------------------------|
| Groups  | n | Range | Index |
| Control (p.o) | 9 | 34-79 | 58 |
| rhEGF I (30µg, p.o) | 9 | 6-69 | 53 |
| rhEGF II (60µg, p.o) | 9 | 25-59 | 46 |
| rhEGF III (120µg, p.o) | 9 | 0-26 | 11 |
| rhEGF IV (60µg, s.c.) | 9 | 0-29 | 19 |
| Cimetidine (p.o) | 7 | 32-52 | 39 |

| Table 2 | The levels of serum EGF in all groups (ng/L) |
|---------|------------------------------------------|
| Groups  | Range | n | Frange |
| Control | 0.51±0.72 | 9 | 0.6±0.09 |
| rhEGF I | 6-69 | 9 | 53 |
| rhEGF II | 25-59 | 9 | 46 |
| rhEGF III | 0-26 | 9 | 11 |
| rhEGF IV | 0-29 | 9 | 19 |
| Cimetidine | 31-52 | 7 | 39 |

| Table 3 | The values of serum gastrin in all groups (ng/L) |
|---------|------------------------------------------|
| Groups  | n | Range | n | Index |
| Control | 40-112 | 6 | 75±28 |
| rhEGF I | 50-160 | 7 | 83±39 |
| rhEGF II | 46-139 | 7 | 93±43 |
| rhEGF III | 49-170 | 7 | 80±42 |
| rhEGF IV | 81-283 | 6 | 149±38 |
| Cimetidine | 18-101 | 6 | 49±28 |

**DISCUSSION**

EGF is a 53-aminoacid peptide isolated for the first time from male mouse salivary glands by Cohen et al in 1962. Later it was also found in submandibular and duodenal Brunner’s glands. Recent studies demonstrated that EGF is a powerful mitogen which is capable of promoting DNA, RNA and protein synthesis and inhibiting gastric acid secretion. The effect of EGF on gastric secretion and cell proliferation suggests that EGF could be responsible for maintaining the structural integrity of the gastrointestinal mucosa and preventing mucosal injury by noxious agents.

This study observed gastric cytoprotection of rhEGF at various dosage, orally or subcutaneously. The degree of injury in rat stomach was significantly less severe in rhEGF group than in control group.

The protective effect of rhEGF is dose-dependent at the range of 60µg·kg⁻¹·d⁻¹ - 120µg·kg⁻¹·d⁻¹. The result suggested that home-made rhEGF can protect gastric mucosa against ethanol injury. Besides, the concentration of rat serum EGF was related to the gastric cytoprotection effect. EGF is an effective protective factor of gastrointestinal epithelia.

Cytoprotective action was first claimed by Jacobson et al in the late 70s. From then on some brain intestinal peptides, such as gastrin, somatostatin, etc. were found to have a similar role in preventing injury from noxious agents. EGF belongs to another kind of substance eliciting the same protection of gastric mucosa. Previous studies had showed that its cytoprotective action was not mainly accomplished through inhibition of gastric secretion. Experimental study in animals showed that EGF could ameliorate acute gastric injury caused by aspirin or stress by small non-antisecretory dosage to stomach. Extrusion of submandibular glands will lower the EGF levels in the gastrointestinal tract markedly by more than 80%, with significant reduction of DNA and RNA contents of the gastric mucosa thus rendering gastric mucosa more susceptible to ulcerogenic agents. Exogenous EGF given parenterally or orally at doses that would stimulate the growth of gastroduodenal mucosa, enhanced ulcer healing in rats with intact salivary glands and could completely reverse the delay in ulcer healing in sialoadenectomized animals. So the gastric protective action of EGF was chiefly related with increase of cellular DNA, RNA and protein synthesis.

This study also showed that a similar dosage of rhEGF when given orally or subcutaneously had different cytoprotective effect. The protection of gastric mucosa from ethanol induced injury in subcutaneous group was significantly greater than oral group, and serum EGF and gastrin levels were also higher in the former group. Our results suggested that cytoprotection of EGF might also be related to gastrin level besides serum EGF level.

In conclusion, rhEGF plays a significant role in the protection of gastric mucosa from ethanol induced injury. Its effect is dose-dependent at a dose range of 60µg·kg⁻¹·d⁻¹ - 120µg·kg⁻¹·d⁻¹. rhEGF might be a new endogenous drug for the treatment of peptic ulcer.

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