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• GASTRIC CANCER •

Preventive effect of hydrotalcite on gastric mucosal injury in rats induced by taurocholate

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Abstract

AIM: To study the preventive effect of hydrotalcite on gastric mucosal injury in rat induced by taurocholate, and to investigate the relationship between the protective mechanism of hydrotalcite and the expression of trefoil factor family 2 (TFF2) mRNA and c-fos protein.

METHODS: Forty five male Wistar rats were randomly divided into hydrotalcite group, ranitidine group and control group. Gastric mucosal injury was induced by introgastric acidified taurocholate. OD value of TFF2 mRNA expression in gastric mucous cells was determined by hybridization and computer image analysis system. OD value of c-fos protein expression in gastric mucous cells was measured by immunohistochemistry and computer image analysis system.

RESULTS: The gross mucosal injury index in hydrotalcite group was significantly lower than that in ranitidine group and control group (8.60±2.20 *vs* 16.32±4.27, 29.53±5.39; $P<0.05$, $P<0.01$). The expression level of TFF2 mRNA in hydrotalcite group was markedly higher than that in ranitidine group and control group (0.56±0.09 *vs* 0.30±0.05, 0.28±0.03, $P<0.05$). The OD value of c-fos protein in hydrotalcite group was higher than that in ranitidine group and control group (0.52±0.07 *vs* 0.31±0.04, 0.32±0.05, $P<0.05$).

CONCLUSION: Hydrotalcite can protect gastric mucosal injury in rats induced by taurocholate, which may be related to the increased expression of TFF2 and c-fos protein.

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INTRODUCTION

Hydrotalcite is one kind of protective agents for gastric mucosal[1,2], it neutralizes the gastric acid[29], stimulates the synthesis of prostaglandin and the release of epidermal growth factor[3] from gastric mucosa[4]. Because hydrotalcite binds to cholalic acid in the stomach[5,6], it is effective on bile reflux gastritis. Trefoil factor family 2 (TFF2)[7-9] is one of the members in the trefoil peptide factor family[10-13] mainly produced by mucus-secreting cells in the gastrointestinal tract[1,14,15]. It involves restitution of epithelial lining after epithelial cell injury[16,17], mucosal defense[18,19] and healing of ulcer[20-23]. c-fos gene[24,25] is one of the earlier expressed genes after gastric mucosa injury. c-fos protein relates to mucosal repair after mucosal injury[26]. In the present study, we aimed to study the preventive effect of hydrotalcite on gastric mucosal injury in rat induced by taurocholate and the relationship between the protective mechanism of hydrotalcite and the expression of TFF2 mRNA and c-fos protein.

MATERIALS AND METHODS

Methods

Animals model Forty-five adult male Wistar rats weighing 200-250 g were divided into three groups randomly: hydrotalcite group, ranitidine group and control group, 15 rats in each group. The animals were housed at the Experimental Animal Center of Wuhan University. Taurocholate was dissolved in normal saline and HCl was added to a final concentration of 0.2 mol/L with pH value of 1.4[27]. The rats in hydrotalcite group were given 100 mg/kg hydrotalcite. The rats in control group were given 1.5 ml normal saline at the same time. The rats in ranitidine group were given 30 mg/kg ranitidine twice 12 hrs the day before. After one hour of hydrotalcite administration, gastric mucosal damage in three groups was induced by introgastric administration of 1.5 ml taurocholate[1] at 15 mmol/L. Two hours later, the animals were killed by cervical dislocation. The abdomen was opened, and the stomach was removed and incised along the greater curvature. The mucosal surface was gently washed with normal saline. Gastric lesions were scored by a previously described scoring system[28] as follows: one point, point erosion; two point, <1 mm of erosion; three point, 1-2 mm of erosion; four point, 3-4 mm of erosion; five point, >4 mm of erosion. The mucosa injury index was calculated on the totally accumulated points. After scored, the mucosa was obtained and prepared for histological examination and other tests.

TFF2 mRNA *in situ* hybridization Gastric mucosa tissue immersion-fixed in neutral buffered formaldehyde was dehydrated, oriented in cross section and embedded in wax. Four micrometer sections were cut, dewaxed, and rehydated to PBS. Sections were permeabilized with proteinase K, postfixed in 4 % paraformaldyhyde in PBS, and acetylated with acetic anhydrated in 0.1 mol/L triethanolamine. The tissues were then dehydrated for hybridization. Hybridization was performed according to the instructions of text kit (Sigma Co). Oligo-nucleo tides probe sequence was 5'-GTAGTGACAAATCTTCCACAGA. The optic density (OD) value of the hybridization signals was assessed by image analysis system.

Immunohistochemistry of c-fos protein Sections of gastric mucosa tissues were incubated with monoclonal antibody against human c-fos protein for four hours at 37 ℃. Immunostaining of c-fos protein was revealed using a commercially available peroxidase-based method (SP vectastain, Zhongshan Co.) according to the instructions of the manufacturer. The optic density of immunosignals was determined by image analysis system.

RESULTS

Gross inspection showed that there were obvious hyperemia, edema, sheet or strip of necrosis and spot hemorrhage in the gastric mucosa of control groups. There were milder lesions after hydrotalcite pretreatment, and microscopic examination confirmed marked protection against taurocholate-induced gastric injury. The degree of lesion in ranitidine group fell in-between the two groups. The gastric mucosa injury index was 8.60±2.20, 16.32±4.27, 29.53±5.39 in hydrotalcite, ranitidine and control groups, respectively. The gastric mucosa index in hydrotalcite group was significantly lower than that in ranitidine group ($P<0.05$) and control group ($P<0.01$).

In all three groups TFF2 mRNA was expressed in the atrium of stomach as revealed by *in situ* hybridization. TFF2 mRNA was mainly confined in gastric epithelial cells and gastric gland mucous neck cells, especially in the margin of the injury region (Figure 1-2).

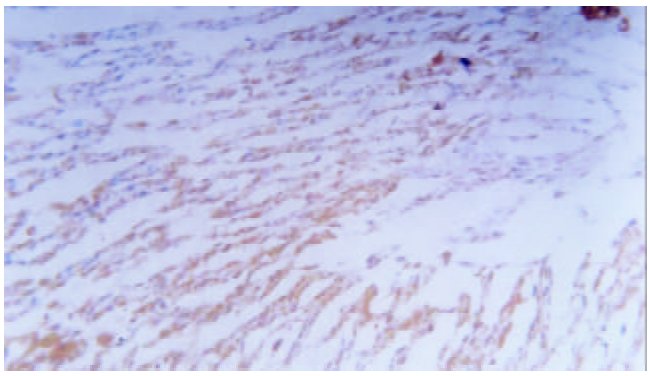

Figure 1 TFF2 mRNA in situ hybridization in hydrotalcite group Positive cells were stained brown-yellow ×200.

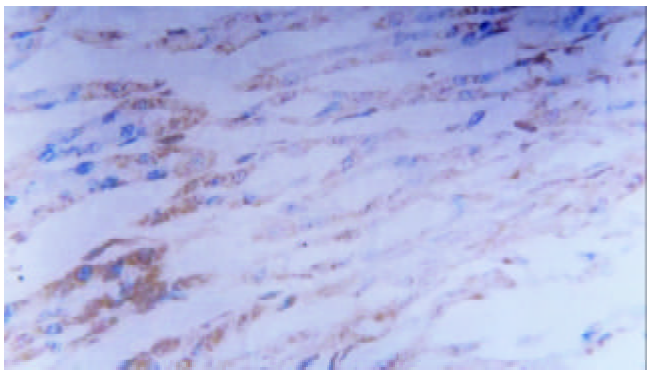

Figure 2 TFF2 mRNA in situ hybridization in hydrotalcite group Positive cells were stained brown-yellow ×400.

Immunochemistry showed that c-fos protein localized in nuclei of positively stained cells, which were mainly gastric gland mucous neck cells (Figure 3-4). The OD values of TFF2 mRNA and c-fos protein in the three groups are shown in Table 1.

Table 1 OD values of TFF2 mRNA staining and c-fos protein staining

Group	Animal number	TFF2 mRNA	c-fos protein
Hydrotalcite	15	0.56±0.09[a]	0.52±0.07[a]
Ranitidine	15	0.30±0.05[b]	0.31±0.04[b]
Control	15	0.28±0.03	0.32±0.05

[a]$P<0.05$ *vs* control, [b]$P>0.05$ *vs* control.

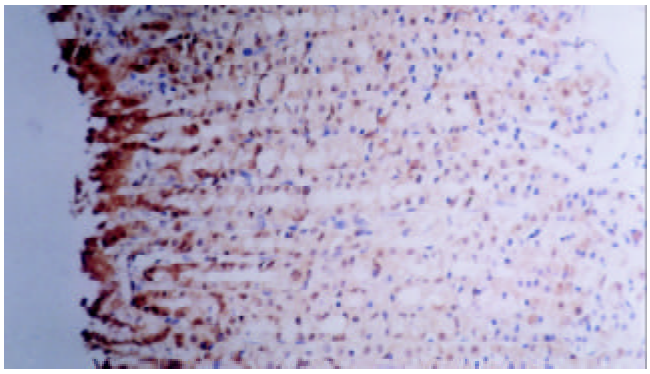

Figure 3 c-fos protein Immunostaining in hydrotalcite group Nuclei of positive cells were stained brown-yellow ×200.

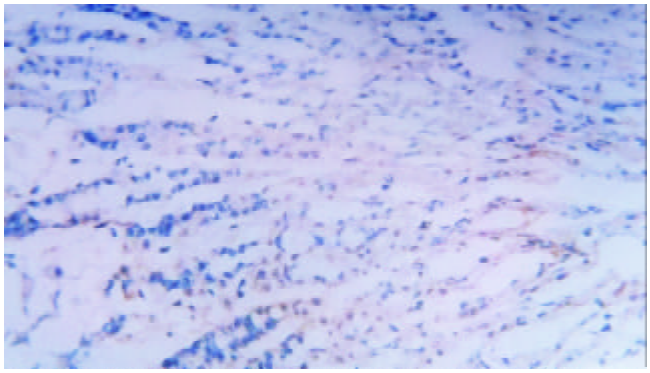

Figure 4 c-fos protein Immunostaining in ranitidine group Nuclei of positive cells w ere stained buff ×200.

DISCUSSION

Hydrotalcite is a complex comprised of aluminum hydroxide, magnesium hydroxide, carbonate and H_2O, which neutralizes gastric acid[29], binds pepsin[30,31] and choliac acid[5,31], *etc*. Our study showed that hydrotalcite could markedly protect gastric mucosa against taurocholate-induced lesions. The mucosal injury index in hydrotalcite group was significantly lower than that in ranitidine and control groups. Choliac acid has been thought to elicit gastric injury by degrading the difference of potential in gastric mucosa, increasing inversed diffusion of acid, and stimulating mast cell to release histamine. Recent researches indicated that transforming growth factor was related to the recovery of gastric mucosal injury induced by taurocholate[27,32,33]. Hydrotalcite has been shown to enhance the synthesis and release of prostaglandin and to increase the amount of epidermal growth factor in mucosal blood[4].

The mechanism of the protective and healing effect of TFF2 on gastric mucosa is still not fully elucidated. *In vitro* studies, TFF2 was shown to stimulate cell migration[34]. Recently hTFF was shown to decrease proton permeation through interacting with mucus in both *in vivo* and *in vitro* studies[35]. Orally-administrated TFF2 was found to bind to the mucus layer of the stomach[36,37], which accelerates the healing of gastric ulcer in the rat[38-41]. In this study, we have shown that hydrotalcite reduces gastric injury induced by taurocholate in rats, and it also increases expression of TFF2 mRNA in margin of the injury region, suggesting that the protective effect of hydrotalcite on gastric mucosa is related to the increase of TFF2 expression. The reason why hydrotalcite increases expression of TFF2 is not yet fully understood. Previous studies indicate that TFF expression and secretion are regulated by neuropeptides and acetylcholine[42], and hydrotalcite increases the amount of epidermal growth factor in gastric mucosa[4]. It is presumed

that hydrotalcite regulates TFF2 mRNA expression through the increase of epidermal growth factor.

C-fos gene is one of the immediate early genes which are involved in the control of cell proliferation in a variety of cell types[43-46]. Change of c-fos mRNA expression is found as early as two hours in the healing of gastric mucosal stress ulcer[26,47]. In this study, the expression of c-fos protein was found two hours later in gastric mucosal damage induced by taurocholate. The amount of c-fos protein in hydrotalcite was higher than that in ranitidine and control groups, suggesting that c-fos protein participates in the mechanism of the protective effect of hydrotalcite in gastric mucosa. It has been shown that EGF[48,49] stimulates the proliferation[50] of cells derived from gastric fundus and induces the expression of c-fos and c-myc, and hydrotalcite increases the amount of epidermal growth factor in gastric mucosa. It may be presumed that hydrotalcite increases c-fos protein through the increase of the amount of epidermal growth factor in gastric mucosa.

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