Protective effect of omeprazole on gastric mucosal of cirrhotic portal hypertension rats

Wei Gao[1], Hai−Ying Li[1], Li−Xin Wang[1], Li−Jun Hao[1], Jian−Li Gao[1], Rong−Juan Zheng[1], Chun−Jiang Cai[2*], Yan−Ling Si[1*]

[1]Department of Gastroenterology, Tangshan Workers´ Hospital, Tangshan 063000, China
[2]Department of Spleen and Stomach, Tangshan TCM Hospital, Tangshan 063003, China

ARTICLE INFO

ABSTRACT

Objective: To observe the protective effect of omeprazole on gastric mucosal of cirrhotic portal hypertension rats. **Methods:** All rats were randomly divided into normal control group, cirrhosis and treatment group. Thioacetamide was used to establish rat model of cirrhotic portal hypertension. The necrotic tissue of gastric mucosa ulcer focus, degree of neutrophils infiltration at the ulcer margin, portal pressure, portal venous flow, abdominal aortic pressure, abdominal aortic blood flow at front end, gastric mucosal blood flow (GMBF), glycoprotein (GP) of gastric mucosa,basal acid secretion, H^+ back −diffusion, gastric mucosal damage index, NO, prostaglandin E_2 (PGE_2) and tumor necrosis factor− α (TNF− α) were determined respectively, and the pathological changes of gastric mucosa were also observed by microscope. **Results:** Compared with cirrhosis group and the control group, the ulcer bottom necrotic material, gastric neutrophil infiltration and UI of the treatment group were all decreased significantly (*P*<0.01), GMBF value, GP values, serum NO, PGE_2, TNF− α were all significantly increased. **Conclusions:** Omeprazole has an important protective effect on gastric mucosal and it can increase gastric mucosal blood flow and related to many factors.

1. Introduction

Portal hypertension gastropathy (PHG) of cirrhotic has become a main clinical manifestations of the decompensated cirrhosis in clinical. It threatens life because of the high incidence of gastric mucosal bleeding. Omeprazole is one of the most common drugs to treat gastric mucosal lesion. Its significant inhibition effect on the gastric acid secretion has been very clear, but the protective effect on gastric mucosal is still unclear. This study is to explore the protective effect of omeprazole on gastric mucosal of cirrhotic portal hypertension rats, and the possible mechanisms.

2. Materials and methods

2.1. Animals and grouping

A total of 30 6−week−old male SD rats were selected, weighting 200−220 g, which were purchased from Experimental Animal Department, XX University. The animals were randomly divided into three groups, including the normal control group (*n*=10), cirrhosis group (*n*=10), treatment group (*n*=10). If rats died or abandoned during the study, they were supplemented again. All rats were fed by standards pellet then kept under constant humidity and temperature

2.2. Reagents and instruments

0.03% thioacetamide (TAA), omeprazole capsules 20 mg

*Corresponding author: Yan−Ling Si, Chief Physician, No. 27 Wenhua Road, Tangshan, Hebei, China.
   E−mail: yanlingemail@163.com
   Tel: 13831554317
   Chun−Jiang Cai, Chief Physician, Department of Spleen and Stomach, Tangshan TCM Hospital, Tangshan 063003, China.
   E−mail: Caichj326@sina.com
   Tel: 13613339968

were purchased from Haikou Qili Pharmaceutical Co., Ltd. Rat prostaglandin E_2 (PGE$_2$), tumor necrosis factor– α (TNF– α) ELISA kit were purchased from Wuhan Boster Reagent Company. Optical microscopy, laser doppler flowmeter, digital millivoltmeter, electronic scales, micro adding sample appliance, *etc*. were provided by the laboratory.

2.3. Model establishement

Rats in the normal control group received 25 mg/kg ketamine under intraperitoneal anesthesia, draped in a sterile manner. They were opened layers by layers and the left suprarenal vein was isolated without special intervention. After adequate hemostasis, they were sutured layers by layers. Animals received water after operation. Rats in the cirrhosis group and treatment group underwent double ligation after left suprarenal vein fully exposed. If there was a branch of small blood vessels, the vessels were also ligated together, then were sutured after hemostasis. The rats received concentration of 0.03% TAA solution as drinking water. Body weight was monitored, and was maintained between 200–260 g. If the margin of body weight increase was more than 20 g or decrease more than 10 g in one week, then concentration of TAA was increased or decreased by 50%. All rats were continuously fed for 14 weeks, then the treatment was stopped for two weeks. After three days of molding, the treatment group were fed by omeprazole 15 mg/kg • d one time everyday. The sampling was performed after 2 weeks.

2.4. Indexes observation

2.4.1. Hemodynamic index detection

Using laser doppler flowmeter, free portal pressure (FPP), portal venous flow (PVF), abdominal aortic pressure (AAP) and abdominal aorta blood flow (AAF) at the beginning point was measured. Greater and lesser curvatures of gastric body, greater and lesser curvatures of gastric antrum on the surface of gastric mucosa were also measured. The average value were obtained as gastric mucosal blood flow (GMBF).

2.4.2. Glycoprotein (GP) of gastric

Mucus were scraped on the surface of mucosa in gastric gland area and weighed. Glycoprotein content was measured by Coomassie brillient blue method.

2.4.3. Basal acid secretion (BAS)

Gastral cavity was washed with normal saline. The beginning part of the duodenum was intubated and the duodenum was ligated. One mL/min saline was added with uniform injection pump, the remote casing was open once every 10 mins for 6 times. H$^+$ concentration was measured by

0. 2 mol/L NaOH microtitration and the average value were obtained.

2.4.4. H$^+$ back diffusion (H$^+$ BD)

Gastral cavity was washed with sarfeh solution (100 mmol/L HCl and 50 mmol/L NaCl). The duodenum was ligated. Sarfeh solution was injected at 3 mL/times, 20 min/times for 3 times. H$^+$ concentration was also measured by microtitration. The value was obtained by minusing Sarfeh fluid H+ concentration.

2.4.5. Index of gastric mucosal lesion

Stomach tissue was removed, cut and flatted.The score was calculated by Guth standard. Spot–like erosions was 1, erosion <1 mm was 2, erosion between 1 –2 mm was 3, erosion between 2–4 mm was 4, erosion> 4 mm was 5.

2.4.6. Histological observation

The gastric tissues were fixed, paraffin routinely embedded, sectioned, and HE stained. Necrotic status and neutrophil infiltration was observed under light microscope, Judgement standard was as follows: No necrotic material or neutrophil infiltration was 0; A few necrotic material and neutrophil infiltration at the bottom edge of the ulcer was 1; Thick layer of necrotic at the ulcer floor and obvious neutrophil infiltration at the marginal tissue of ulcer was 3; Between them was 2.

2.4.7. Serum NO, PGE$_2$, TNF– α measurment

Venous blood samples were collected before the rats were sacrificed. After centrifugation, the serum NO content was measured. The serum PGE$_2$, TNF– α levels were measured by radioimmunoassay.

2.5. Statistical analysis

The data was analyzed with SPSS 13.0 software. Data were expressed as mean±SD. Homogeneity of variance was carried out for the measurement material, and multiple comparison was used if there is significant differences. $P < 0.05$ was considered as statistical significance.

3. Results

3.1. Mucosa observation

The gross specimen of gastric in normal control rats showed smooth and complete surface. Microscope showed glands arranged regularly. Gross specimen of cirrhotic rats gastric showed obvious hyperemia and edema, one or more strip–shaped erosion and hemorrhagic focus, necrotic

material. Microscope showed glandular arranged in disorder, great number of neutrophils infiltration, even involving in the serous layer. Gross specimen of gastric mucosal in the treatment group was with more complete structure. It also showed some hemorrhagic spot or hemorrhagic focus, but was decreased significantly than that in the cirrhosis group. Amount of necrotic material at the bottom of the ulcer was also less than that in the cirrhosis group. Microscope showed glandular arranged in order, a little neutrophils infiltration which was less than that of cirrhosis group.

3.2. Gastric mucosal damage index (GMDI)

In the control group, there was no damage; the gastric mucosal damage index of cirrhotic rats was significantly increased. Compared with the control group and treatment group, the differences were significant ($P<0.05$). There was still some gastric mucosal injury in the treatment group, the damage index was significantly higher than the control group ($P<0.05$) (Table 1).

3.3. Hemodynamic score results

FPP and AAF index of the cirrhosis group and treatment group were significantly higher than that in the control group, which indicated successful cirrhosis model. The gastric GMBF of the cirrhosis group decreased significantly compared with the control group and the treatment group ($P<0.05$). The gastric GMBF of the treatment group was slightly lower than the normal group, but the difference had no statistically significant ($P>0.05$) (Table 2).

3.4. Measurement of mucosal glycoproteins and H^+

GP in Cirrhosis group was significantly lower than the control group and treatment group ($P<0.05$). There was no significantly difference in BAS secretionamong three groups ($P>0.05$) (Table 3).

3.5. Serum NO, PGE_2, TNF–α of humoral factors

PGE_2 expression in the cirrhosis group and the treatment group was decreased significantly compared with that in the controls ($P<0.05$); There was no significantly difference between the treatment group and the cirrhosis group ($P>0.05$). TNF–α expression in the cirrhosis group was significantly increased compared with that in the control group and the treatment group, ($P<0.05$); TNF–α expression

Table 1
Gastric mucosal lesion.

Groups	Number of cases	Damage index	Necrotic tissue	Infiltration of neutrophils
Control group	10	0	0	0
Cirrhosis group	10	38.5±4.2[*]	2.8±1.0[*]	3.4±0.5[*]
Treatment group	10	8.8±1.1[*△]	1.3±0.6[*△]	1.6±0.6[*△]

Note: * Compared with control group, $P<0.05$, △ Compared with cirrhosis group, $P<0.05$.

Table 2
Hemodynamic parameters.

Groups	FPP (kPa)	PVF (mL/min)	AAP (kPa)	AAF (mL/min)	GMBF (V/mV)
Control group	0.8±0.2	14.3±2.2	14.3±1.3	37.9±4.3	305.3±22.5
Cirrhosis group	1.5±0.4[*]	13.2±2.0	15.1±1.0	78.4±7.6[*]	179.6±18.7[*]
Treatment group	1.5±0.3[*]	13.6±2.1	15.3±1.2	79.5±6.8[*]	288.7±20.1[△]

Note: * Compared with control group, $P<0.05$, △ Compared with cirrhosis group, $P<0.05$.

Table 3
Glycoproteins and H^+BD of gastric mucosal.

Groups	GP (mg)	BAS (μ Eq/ h)	H^+BD (μ Eq/h)
Control group	0.4±0.2	10.8±1.1	40.7±4.2
Cirrhosis group	0.2±0.1[*]	9.8±1.4	133.8±6.9[*]
Treatment group	0.4±0.2[△]	10.3±1.2	44.4±5.6[△]

Note: * Compared with control group, $P<0.05$, △ Compared with cirrhosis group, $P<0.05$.

Table 4
NO, PGE_2 and TNF–α .

Groups	Number of cases	NO (μ mol/L)	PGE_2 (mol/L)	TNF–α (pg/mL)
Control group	10	9.3±2.9	354.8±38.5	0.4±0.1
Cirrhosis group	10	27.6±4.1[*]	262.4±30.6[*]	1.7±0.7[*]
Treatment group	10	40.4±5.3[*△]	274.2±32.2[*]	1.0±0.3[*△]

Note: * Compared with control group, $P<0.05$, △ Compared with cirrhosis group, $P<0.05$.

in treatment group was significantly higher than the control group ($P<0.05$). NO expression in the treatment group was significantly higher than that in the control group and the cirrhotic group ($P<0.05$); NO expression in the cirrhotic group was significantly higher than the control group ($P<0.05$) (Table 4).

4. Discussion

Cirrhosis is the late stage of various chronic liver diseases, portal hypertension is the main manifestation during this stage and all the complications have a severe influence on patients 'quality of life. Therefore, it becomes a research hotspot to protect the gastric mucosa under portal hypertension. According to the previous studies, abundant blood flow is the basis for maintaining normal mucosal function in the physiological state. After the formation of portal hypertension, a large number of visceral blood flow through the arteriovenous shunt and collateral circulation directly into the systemic circulation, makes mucosal capillary network in the ischemic state, which may lead to the occurrence of PHG. Omeprazole is one of the most important drugs as a proton pump inhibitor, how to reduce stomach acid secretion and protect the gastric mucosa became the focus of this study.

To observe whether omeprazole treatment have an impact on gastric mucosal ulceration, some studies by established an animal model of acute gastric mucosal injury. The result showed that omeprazole treatment has an important effect for acute gastric mucosal injury, and large doses are better than small doses, and that the proton pump inhibitors omeprazole can inhibit gastric acid secretion, H^+ diffused from gastric parietal cells to mucosa significantly reduced, prompting the gastric PH value increased significantly. This study suggests that H^+ BD significantly reduced, which is consistent with the above results. Omeprazole can significantly reduce mucosal cells acidification damage degree. Meanwhile, the study suggests that GP protein was significantly increased. GP as a glycoprotein of epithelial cells cover the mucosal surface and buffer the mechanical damage between the stomach, while also provide the attachment platform for the gastric mucosal barrier. It is considered that GP is an accurate and sensitive indicator which can reflect the gastric mucosa and also play an important role in anti-acid, resisting pepsin and prevent ulceration. GP's increase can help to protect the integrity of the gastric mucosa. Studies suggest that during gastric mucosal injury, the GP decrease degree is consistent with the mucosal injury. Proton pump inhibitor can protect mucosa and benefit to the epithelial repair.

Animal experiments suggested that NO as an important radical in the blood can change the gastric mucosal blood flow, and the use of NOS inhibitors will increase gastric mucosal injury of animal. The increase of serum NO will lead to the increase of serum NOS and iNOS, and also reduced cNOS, thereby damage to the tissue. But there are also studies suggested that after the use of PPI drugs, gastric mucosal protective level was positively correlated with the NO, considered the mechanism is related to the influence of parietal cells H^+–K^+–ATP activity. This study suggests that NO of the omeprazole treatment group was significantly higher and the GMBF also increased. Maybe the NO vasodilatory effects can promote the increase of GMBF. Although NO free radical will damage the gastric mucosa tissue, NO may mediate the omeprazole's protective effect on gastric mucosa after omeprazole inhibited H^+–K^+–ATP enzyme.

PGE_2 is one of the major PGs of gastric, but experiments suggest that it can inhibit the apoptosis of rat gastric mucosa. It can increase the secretion of gastric epithelial mucus and the bicarbonate, also protect gastric mucosa to avoid any attack by various invasion factors, and have the same damage effect on the acid–ethanol. This study suggests that PGE_2 significantly lower in cirrhotic rats, but the omeprazole treatment did not change significantly. TNF–α as one of the inflammatory cytokines can strengthen tail lesions by increasing vascular permeability during the PHG. Studies suggest that TNF–α content was significantly associated with the damage degree of toxic mucosal. It play an important role as the media factor of endotoxin–induced gastric mucosal injury, suggested that it can be used as early monitoring indicators for the clinical effect of gastric mucosal injury. This study suggests that the TNF–α significantly increased in the cirrhosis group, but it can be effectively reduced after drug treatment. It may be due to the increase of TNF–α can increase the local tissue inflammation, leading to mucosal ischemia and hypoxia, decreased gastric mucosal blood flow and increased blood sedimentation, and then increased the damage. This study suggests that the damage degree of gastric mucosal surfaces can be significantly reduced after omeprazole treatment, possibly because of many factors mentioned above.

In summary, we can speculate that omeprazole play a protective role in the gastric mucosa by improving the gastric environment, through the influence of inflammatory cytokines to slowdown its further damage the gastric mucosa. These results suggested that omeprazole have a protective effect on the cirrhotic portal hypertension induced gastric mucosal injury.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Thissen JP. How proinflammatory cytokines may impair growth and cause musclewasting. *Horm Res* 2007; **67**(Suppl 1): 64–70.

[2] Ma X, Liu BG. New progress in portal hypertension drugs. *World J Digestol* 2008; **16**: 3775–3781.

[3] Reynaert H, Urbain D, Geerts A. Regulation of sinusoidal perfusion in portal hypertension. *Anat Rec (Hoboken)* 2008; **291**: 693–698.

[4] Zou WL, Yang Z, Zang YJ, Li DJ, Liang ZP, Shen ZY. Inhibitory effects of prostaglandin E1 on activation of hepatic stellate cells in rabbits with schistosomiasis. *Hepatobiliary Pancreat Dis Int* 2007; **6**: 176–181.

[5] Yang WY, Wu JX, Dai Q, Jiang FF, Zhao LM, Zhang YJ, et al. Long–term Mr Octreotide on portal hypertension of liver fibrosis in rats. *World Chin J Digestol* 2008; **16**: 2815–2819.

[6] Tu CT, Guo JS, Wang M, Wang JY. Antifibrotic activity of rofecoxib in vivo is associated with reduced portal hypertension in rats with carbon tetrachloride–induced liver injury. *J Gastroenterol Hepatol* 2007; **22**: 877–884.

[7] Fan X, Xu Y, Zhang HL. Prostaglandin E2 and omeprazole in treating poisoning related acute mucosal lesion. *J Central South Univ (Med)* 2009; **34**(10): 1036–1041.

[8] Brzozowski T, Konturek PC, Konturek SJ, Pajdo R, Kwiecien S, Pawlik M, et al. Ischemic preconditioning of remote organs attenuates gastric ischemia–reperfusion injury through involvement of prostaglandins and sensory nerves. *Eur J Pharmacol* 2004; **499**(1/2): 201–213.

[9] Watanabe T, Higuchi K, Hamaguchi M, Shiba M, Tominaga K, Fujiwara Y, et al. Monocyte chemotactic protein–1 regulates leukocyte recruitment during gastric ulcer recurrence induced by tumor necrosis facto–ralpha. *Am J Physiol Gastrointest Liver Physiol* 2004; **287**(4): G919–G928.

[10] Spyridon L, Akira N, Hiromasa K, Katsutoshi G, Takao M, Yoshiki O, et al. The development of the endothelin–1–induced gastric ulcer: time sequence analysis of morphologic changes. *Int J Exp Pathol* 1994; **75**(5): 345–355.

[11] Luo JC, Shin VY, Yang YH, Wu WKK, Ye YN, So WHL, et al. Tumor necrosis factor–alpha stimulates gastric epithelial cell proliferation. *Am J Physiol Gastrointest Liver Physiol* 2005; **288**(1): G32–G38.

[12] Duan YM, Li ZS, Zhan XB, Xu GM, Tu ZX, Gong YF. Changes in endothelin–1 gene expression in the gastric mucosa of rats under cold–restrain–t stress. *Chin J Dig Dis* 2004; **5**(1): 28–34.

[13] Gyires K. Gastric mucosal protection: from prostaglandins to gene–therapy. *Curr Med Chem* 2005; **12**(2): 203–215.

[14] Tarnawski a S. Cellular and molecular mechanisms of gastrointestinal ulcer healing. *Dig Dis Sci* 2005; **50**(Suppl 1): S24–S33.

[15] Nakazawa K, Narumi Y, Ishikawa S, Yokoyama K, Nishikage T, Nagai K, et al. Effect of prostaglandin E1 on inflammatory responses and gas exchange in patients undergoing surgery for oesophageal cancer. *Br J Anaesth* 2004; **93**: 199–203.

[16] Singal AK, Ahmad M, Soloway RD. Duplex Doppler ultrasound examination of the portal venous system: an emerging novel technique for the estimation of portal vein pressure. *Dig Dis Sci* 2010; **55**: 1230–1240.

[17] Hennenberg M, Trebicka J, Sauerbruch T, Heller J. Mechanisms of extrahepatic vasodilation in portal hypertension. *Gut* 2008; **57**: 1300–1314.

[18] Gatta A, Bolognesi M, Merkel C. Vasoactive factors and hemodynamic mechanisms in the pathophysiology of portal hypertension in cirrhosis. *Mol Aspects Med* 2008; **29**: 119–129.

[19] Sanyal AJ, Bosch J, Blei A, Arroyo V. Portal hypertension and its complications. *Gastroenterology* 2008; **134**: 1715–1728.

[20] Reynaert H, Rombouts K, Vandermonde A, et al. Ex2 pression of somatostatin receptors in normal and cirrhotic human liver and in hepatocellular careinoma. *Gut* 2004: **53**(8):1180–1189.

[21] Cot GA, Rice JP, Bulsiewicz W, et al. Use of physician education and computer alert to improve targeted use of gastroprotection among NSAID users. *Am Am J Gastroenterol* 2008; **103**(5): 1097–1103.

[22] Jiang GR, Zhang LR, Wang CX, Liang GQ, Ge HN. Effect of Yiqihuoxue decoration combined with omeprazole on quality of ulcer healing in rats. *J Southeast Univ* 2013; **32**(3): 320–323.

[23] Wei J, Zhai JS. Peptic ulcer clinical analysis. *Modern Med* 2010; **38**(1): 72–73.

[24] Fang HM, Wang JJ, Zhang LJ, Xu JM, Mei Q, Lu CX, et al. Omeprazole and rabeprazole azole effect on rat intestinal P–glycoprotein. *Chin J Hospital Pharm* 2013; **33**(1): 7–11.

[25] Dahan A, Amidon GL. Segmental dependent transport of low permeability compounds along the small intestine due to P–glycoprotein: the role of efflux transport in the oral absorption of BCS class Ⅲ drugs. *Mol Pharm* 2009; **6**(1): 19–28.

[26] Fang HM, Mei Q, Diao L, Jin J, Chen ML, Xu XH, et al. Omeprazole intestinal first effect and mechanism research. *Chin J Hospital Pharm* 2010; **30**(7): 543–547.

[27] Fang HM, Xu JM, Mei Q, Diao L, Chen ML, Jin J, et al. Involvement of cytochrome P450 3A4 and P–glycoprotein in the intestinal firstpass extraction of omeprazole in rabbits. *Acta Pharmacologica Sinica* 2009; **11**(30): 1566–1572.

[28] Van Waterschoot RA, Schinkel AH. A critical analysis of the interplay between cytochrome P450 3A and P–glycoprotein: recent insights from knockout and transgenic mice. *Pharmacol Rev* 2011; **63**(2): 390–410.

[29] Chen RY, Peng XP, Wang L, Wei XH, Wan YS, Li WJ. Study the protective effects of Omeprazole on cisplatin cause rat stomach injury. *Chinese Pharmacy* 2012; **23**(25): 26–28.

[30] Fan X, Xu Y, Zhang HL. Protective effects of Omeprazole on the toxic acute gastric mucosal lesion in rats. *Chin Clin Pharmacol Ther* 2010; **15**(6): 626–630.