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Original article

The Effects of Cholestasis and Cirrhosis on Gastric Acid and Pepsin Secretions in Rat: Involvement of Nitric Oxide

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Abstract

Objective(s)

The liver has major role in the organism homeostasis, interactions with other systems, synthesis and metabolism of bile production, drug detoxification and hormone inactivation. Cholestasis can be defined as an impairment of the bile flow which can lead to hepatocytes necrosis and finally cirrhosis. Some studies reported a gastric acid secretion reduction in cirrhotic subjects, while others reported normal production gastric acid secretion. Our aim was to evaluate the effects of cholestasis and cirrhosis on gastric acid and pepsin secretions and its possible mechanism in rat.

Materials and Methods

Male Wistar rats were randomly divided into five groups (n= 8): control, cholestasis, sham cholestasis, cirrhosis and sham cirrhosis. Laparatomy was done under general anesthesia and then bile duct ligation (BDL) was performed. After 2 and 4 weeks in cholestasis and cirrhosis groups respectively, gastric content was collected by wash-out technique. Basal and stimulated acid and pepsin secretions were measured by using titration and the Anson method respectively in all groups. In order to measure stimulated acid and pepsin secretions, pentagastrin (25 µg/kg, i.p.) was used. Nitric Oxide (NO) metabolites of gastric tissue were determined by Griess microassy method.

Results

Acid and pepsin secretions were significantly reduced in cholestatic and cirrhotic rats in comparison with control and sham groups ($P< 0.01$). NO metabolite of gastric tissue was significantly increased in cholestatic and cirrhotic rats ($P< 0.01$).

Conclusion

Reducing of gastric acid and pepsin output in cholestatic and cirrhotic rats may be due to increasing in NO content of gastric tissue.

Keywords: Cholestasis, Gastric acid, Liver cirrhosis, Nitric oxide, Pepsin

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Introduction

The liver plays an important role in the synthesis and metabolism of protein, organism homeostasis, glucose and bile production, drug detoxification and hormone inactivation. Therefore, liver diseases, such as cholestasis, can deleteriously affect other systems of the body (1). Cholestasis can be defined as an impairment of the bile flow. The consequences are retention of the bile acids, bilirubin and other cholephils in the liver and blood. As a result, the release of bile acids into the intestine decreases (2). Elevated levels of bile acids in the liver can lead to apoptosis or necrosis of hepatocytes and eventually to cirrhosis (3). Cirrhosis is associated with several circulatory abnormalities like hyperkinetic circulation (4), gastrointestinal bleeding from esophageal varices, ascites, hepato-renal syndrome, jaundice, malnutrition (5, 6) and severe morphologic changes in the intestinal microvilli (7, 8). It also decreases the gastrointestinal motility which is demonstrated by slowed gastric emptying and prolonged gastrointestinal transition time (9, 10). One of the most important effects of cirrhosis is the increased risk of peptic ulcers (11). The role of the cirrhosis in gastric secretion is not well known. Some studies reported a reduction in acid secretion (12, 13) while others reported normal secretion (14-16). The amount of acid and pepsin secretions are also directly related to peptic ulcer disease. Also, it has been reported that N^G –nitro-L-arginine methylester (L-NAME) has protective effect on gastric damage and mucosa secretion in cholestatic rats (17).

Therefore, the main goal of this study was to evaluate the effects of cholestasis and cirrhosis on gastric acid and pepsin secretions, and its relation to NO level in the gastric tissue of rat.

Materials and Methods

The procedures were in accordance with the guideline for the care and use of laboratory animals of Tehran University of Medical Sciences, Tehran, Iran. Male Wistar rats weighting 200-250 g were housed in 4 groups, maintained in 22±2 °C and 12 hr light/12 hr dark cycles and, with free access to food and water. The animals were randomly divided into five groups (n= 8): control, cholestasis, sham cholestasis, cirrhosis and sham cirrhosis. Laparatomy was performed under general anesthesia (ketamine 50 mg/kg and Xylazine 10 mg/kg i.p, Gedeon and Alfasan Companies, respectively). The common bile duct was ligated. After 2 and 4 weeks in cholestasis and cirrhosis groups respectively, main experiment was done. Before performing experiment, animals were deprived of food for 24 hr, but had a free access to water. Then under general anesthesia (Thiopantal sodium 50 mg/kg, i.p., Sandoz Company) tracheotomy and laparatomy was performed. After 30 min recovery, gastric juice was collected by washout technique (The gastric contents were removed and 1ml of sample was titrated by Titrator (DIN, Germany). After measuring the pH, titration was continued up to pH= 7 by adding 0.01N sodium hydroxide. Titratable acid was calculated and reported as micromole acid per 15 min) (18). For measuring basal acid output, 1 ml normal saline solution was entered in to the stomach, after 15 min another 1ml was added for dilution and easier collection, then stomach content was aspirated for acid titration. The remaining gastric juice (1 ml) was used for pepsin measurement by the Anson method (In this method, pepsin acted on the substrate (hemoglobin) and the final product of reaction was measured by UV spectrophotometer (JENWAY 6105 UV/Vis, UK, λ= 280 nm) (18).

In order to measure pantagastrin-stimulated acid and pepsin secretion, pantagastrin (25 µg/kg, i.p. Sigma Company) was used; 15 min after using of pantagastrin, stimulated acid and pepsin secretions were measured (19). For omission of circadian rhythms, experiment was started at 8 am. Gastric tissue was prepared to assay nitric oxide (NO) metabolites by using Griess microassy method (20). The animals were killed by infusion of potassium chloride to the heart at the end of experiments.

All data are expressed as mean±SEM. Statistical analysis of data (NO level in gastric tissue, gastric acid and pepsin output) was evaluated by means of analysis of variance (ANOVA), followed by Tukey's HSD post hoc test. $P< 0.05$ were considered statistically significant.

Results

Plasma bilirubin increased in BDL animal and remained significantly higher than control and sham groups after the 3rd day of study (control= 137.2±3.2, Sham-cholestasis= 136.8±3.14, Sham-cirrhosis= 137.5±3.3, Cholestasis= 167.5±3.6, cirrhosis= 174.2±3.2 µmol/lit, $P< 0.05$) (Figure 1). Two days after laparotomy, BDL rats showed manifestations of cholestasis (jaundice, dark urine).

Basal gastric acid secretion was significantly decreased in cholestatic and cirrhotic (1.82±0.01, 1.47±0.07 µmol/15 min) rats compared to the sham cholestasis and sham cirrhosis (4.21±0.16, 4.13±0.20 µmol/15 min) and control group (4.03±0.12 µmol/15 min) ($P< 0.01$) (Figure 2). Basal gastric acid secretions in the cirrhotic (1.47±0.07 µmol/15 min) group was significantly lower than the cholestatic group (1.82±0.01 µmol/15 min) ($P< 0.05$). In comparison with the basal gastric acid secretion, the pentagastrin stimulated gastric acid secretion showed a significant increase in all groups. However, this increase in cirrhotic (6.42±0.52 µmol/15 min) and cholestatic (7.32±0.56 µmol/15 min) groups was milder than the other groups (control= 11.14±0.98, sham cholestasis= 11.68±0.98, sham cirrhosis= 10.73±0.94 µmol/15 min) ($P< 0.02$) (Figure 2).

The level of NO metabolites of Gastric tissue in cholestatic (69.9±1.70 µm/g/wet.weight) and cirrhotic (75.6±1.50 µm/g/wet.weight) were significantly higher than the remaining 3 groups (sham cholestasis=44.20±1.50, sham cirrhosis= 45.10±1.60 and control group= 46.5±1.80 µm/g/wet.weight) ($P< 0.01$). Moreover, this increase in cirrhotic (75.6±1.50 µm/g/wet.weight) group was significantly higher than the cholestatic group (69.9±1.70 µm/g/wet.weight) ($P< 0.05$) (Figure 3).

In the cholestatic and cirrhotic groups, (0.35±0.05, 0.21±0.03 µg/15 min respectively) there was a significant decrease in basal pepsin secretion compared to the sham groups (sham cholestasis=0.68±0.06, sham cirrhosis= 0.62±0.04 µg/15 min) and control (0.70±0.07 µg/15 min) group ($P< 0.01$) (Figure 4). Basal gastric pepsin secretions in the cirrhotic (0.21±0.03 µg/15 min) group was significantly lower than the cholestatic (0.35±0.05 µg/15 min) group ($P< 0.05$). In comparison with the basal gastric pepsin secretion, the pentagastrin stimulated gastric pepsin secretion showed a significant increase. However, this increase in cirrhotic and cholestatic groups (1.08±0.82, 1.14±0.9 µg/15 min respectively) was lower than the other groups (control=2.76±0.24, sham cholestasis=2.56±0.12, and sham cirrhosis= 2.60±0.13 µg/15 min) ($P< 0.02$) (Figure 4).

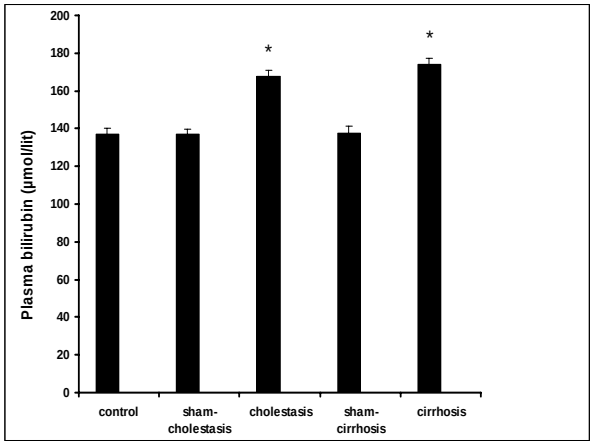

Figure 1. The level of plasma bilirubin in cholestatic, cirrhotic and other groups (Mean±SEM, n= 8). *$P< 0.05$ comparison of cholestasis, cirrhosis and control, sham-cholestasis and sham-cirrhosis.

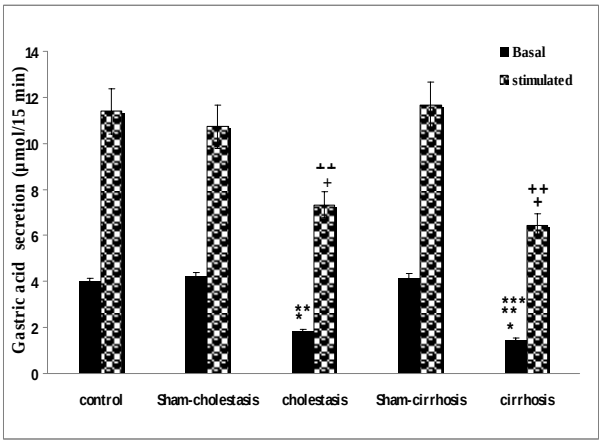

Figure 2. Comparison of gastric acid secretion in basal and stimulated status in 5 groups (Mean±SEM, n=8). *$P< 0.01$ comparison of control, cholestasis & cirrhosis in basal acid secretion.

**$P< 0.01$ comparison of sham-cholestasis, sham-cirrhosis and cholestasis & cirrhosis in basal acid secretion.

***$P< 0.05$ comparison of cholestasis & cirrhosis in basal acid secretion.

$^{+}P< 0.02$ comparison of control, cholestasis & cirrhosis in pentagastrin stimulated acid secretion.

$^{++}P< 0.02$ comparison of sham-cholestasis, sham-cirrhosis and cholestasis & cirrhosis in pentagastrin stimulated acid secretion.

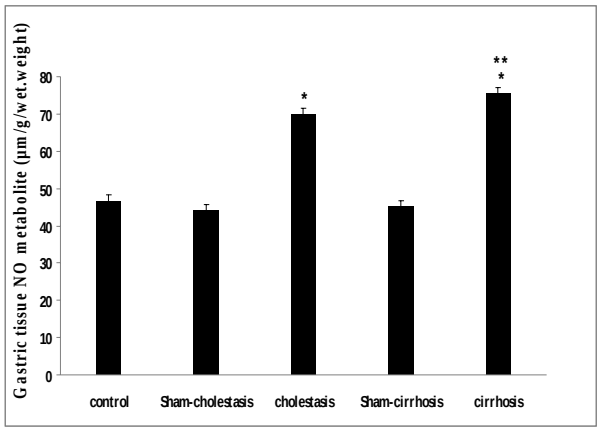

Figure 3. The level of gastric tissue NO metabolites in cholestatic, cirrhotic and other groups (Mean±SEM, n=8).
**P*< 0.01 comparison of control, sham-cholestasis, sham-cirrhosis, cholestasis & cirrhosis in gastric tissue NO metabolite level.
***P*< 0.05 comparison of cholestasis & cirrhosis in gastric tissue NO metabolite level.

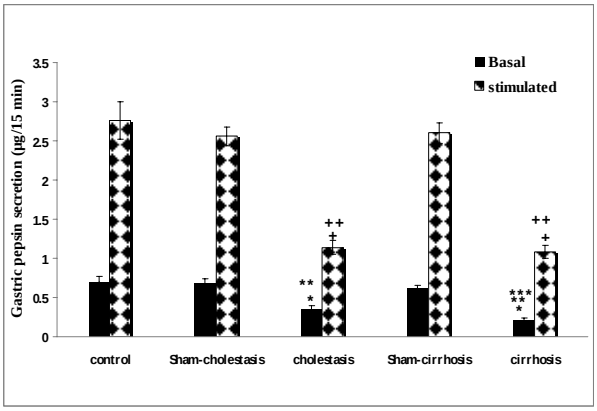

Figure 4. Comparison of gastric pepsin secretion in basal and stimulated status in 5 groups (Mean ±SEM, n=8).
**P*< 0.01 comparison of control, cholestasis & cirrhosis in basal pepsin secretion.
***P*< 0.01 comparison of sham-cholestasis, sham-cirrhosis and cholestasis & cirrhosis in basal pepsin secretion.
****P*< 0.05 comparison of cholestasis & cirrhosis in basal pepsin secretion.
^{+}P< 0.02 comparison of control, cholestasis & cirrhosis in pentagastrin stimulated pepsin secretion.
^{++}P< 0.02 comparison of sham-cholestasis, sham-cirrhosis and cholestasis & cirrhosis in pentagastrin stimulated pepsin secretion.

Discussion

The potent inhibitory effect of cholestasis and cirrhosis on acid and pepsin secretions was confirmed in the present study. Our results showed that cholestasis and cirrhosis can increase the levels of gastric tissue NO metabolites. However, the mechanism by which cholestasis and cirrhosis inhibit acid and pepsin secretions is unknown. The role of gastric secretion in cirrhosis is controversial; some studies reported reduced acid secretion (14, 21) while others reported normal production (12, 22). Previous studies showed that secretion of gastrin hormone that is itself regulated by intragastric pH, regulates the production of HCl and pepsin, and finally is partially metabolized by the liver and kidneys (23). Basal level of serum gastrin is increased in cirrhosis. This increase is secondary to gastric hypoacidity and decrease of inhibitory feedback of gastrin secretion that has an inverse relationship with each other (13). It has been found that patients suffering from cirrhosis have a gastric pH higher than normal and this hypochlorhydria is proportional to cirrhosis intensity (24). Studies about the 24 hr acidity in cirrhotic patient not only showed a marked hypoacidity (25) but also a significant decreased of pepsin release (26). Neuronal NOS (nNOS) is expressed in parietal cells. This finding suggests that endogenous NO, acting as an intracellular signaling molecule, may participate in the regulation of gastric acid secretion. About 50% of the nerves in the enteric nervous system contain nNOS (27). It is likely that NO inhibits vagally-mediated acid secretion by suppressing neuronal activity of the vagus nerve (28). Effects of NO on the inhibitory and stimulatory actions are complex (29, 30).

It should be mentioned that NO has dual effect on gastric acid secretion, i.e. small amounts of NO have a stimulatory effect on gastric ECL cells resulting in increased acid secretion, while large amounts of NO have an inhibitory effect on parietal cells leading to gastric secretion (31, 32).

Conclusion

It can be concluded that the decrease in acid and pepsin secretion in cholestasis & cirrhosis is probably the consequence of altered gastric NO cellular levels.

Acknowledgment

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References

1. Gaskari SA, Honar H, Lee SS. Therapy insight: cirrhotic cardiomyopathy. Nat Clin Pract Gastroenterol Hepatol 2006; 3:329–337.
2. Paumgartner G. Medical treatment of cholestatic liver diseases: From pathobiology to pharmacological targets. World J Gastroenterol 2006; 12:4445-4451.
3. Paumgartner G, Beuers U. Ursodeoxycholic acid in cholestatic liver disease: mechanisms of action and therapeutic use revisited. Hepatology *2002*; 36:525-531.
4. Wong F. Cirrhotic cardiomyopathy. Hepatol Int 2009; 3:294–304.
5. Avila FT, Avila FS, de-Sicilia MS, Chavez-Tapia NC, *et al*. Prevalence of metabolic syndrome, obesity and diabetes type 2 in cryptogenic cirrhosis. World J Gastroenterol 2008; 14:4771–4775.
6. Mesejo A, Juan YM, Serrano A. Liver cirrhosis and encephalopathy: Clinical and metabolic consequences and nutritional support. Nutr Hosp 2008; 23:8-18.
7. Castilla-Cortázar I, Pascual M, Urdaneta E, Pardo J, Puche JE, Vivas B, *et al*. Jejunal microvilli atrophy and reduced nutrient transport in rats with advanced liver cirrhosis: improvement by Insulin-like Growth Factor I. BMC Gastroenterol 2004; 4:12.
8. Conchillo M, Prieto J, Quiroga J. Insulin-like growth factor I (IGF-I) and liver cirrhosis. Rev Esp Enferm Dig (Madrid) 2007; 99:156-164.
9. Wang X, Zhong YX, Zhang ZY, Lu J, Lan M, Miao JY, Guo XG, Shi YQ, Zhao YQ, Ding J, Wu KC, Pan BR, Fan DM. *et al*. Effect of L-NAME on nitric oxide and gastrointestinal motility alterations in cirrhotic rats. World J Gastroenterol 2002; 8:328-332.
10. Schiedermaier P, Harrison P, Arthur M, Grandt D, Sutton R, Drewe J, *et al.* Effect of the Somatostatin Analogue Lanreotide on Meal-Stimulated Portal Blood Flow in Patients with Liver Cirrhosis. Digestion 2002; 65:56-60.
11. Konturek SJ, Gonciarz M, Bielanski W, Mazur W, Mularczyk A, Konturek PC, *et al*. Progastrin and its products from patients with chronic viral hepatitis and liver cirrhosis. Scand J Gastroenterol 2003; 38:643-647.
12. Lodato F, Azzaroli F, Di Girolamo M, Feletti V, Cecinato P, Lisotti A, *et al* .Proton pump inhibitors in cirrhosis: Tradition or evidence based practice? World J Gastroenterol 2008; 14:2980-2985.
13. Konturek SJ, Gonciarz M, Gonciarz Z, Bielanski W, Mazur W, Mularczyk A, *et al.* Progastrin and its products from patients with chronic viral hepatitis and liver cirrhosis . Scand J Gastroenterol 2003; 38:643-647.
14. Kitano S, Dolgor B .Does portal hypertension contribute to the pathogenesis of gastric ulcer associated with liver cirrhosis? J Gastroenterol 2000; 35:79-86.
15. Cortez-pinto H, Ferra MA, Baptista A, Demoura MC, De Moura MC, Portela-Gomes GM l. Serum gastrin and gastrin=immunoreactive cells in the antral mucosa of patients with alcoholic liver disease. APMIS 2000; 108: 51-56.
16. Yeh JL , Peng YC , Tung CF , Chen GH , Chow WK, Chang CS, *et al*. Role of Helicobacter pylori in cirrhotic patients with dyspepsia: $A^{13}C$-urea breathe test study. Adv Ther 2001; 18:140-150.
17. Nahavandi A, Dehpour AR, Mani AR, Homayounfar H, Abdoli A, Abdolhoseini MR, *et al.* The role of nitric oxide in bradycardia of rats with obstructive cholestasis . Eur J Pharmacol 2001; 411: 135–141.
18. Nabavizadeh F , Zahedi Asl S , Garib Naseri MK, Vahedian J. Effects of thyroid hormones on basal and stimulated gastric acid secretion due to histamine, carbachol and pentagastrin in rats. Saudi Med J 2003; 24:341-346.
19. Nabavizadeh F, Salimi E, Sadroleslami Z, Vahedian J**.** Saffron (*Crocus sativus*) increases gastric acid and pepsin secretions in rats: Role of nitric oxide (NO). Afr J Pharmacy Pharmacol 2009; 3:181-184.
20. Nahrevanian H, Gholizadeh J, Farahmand M, Assmar M, Sharifi K, Ayatollahi Mousavi SA, *et al*. Nitric oxide induction as a novel immunoepidemiological target in malaria-infected patients from endemic areas of the Islamic Republic of Iran. Scand J Clin Lab Invest 2006; 66:201-209.
21. Zullo A, Romiti A, Rinaldi V, Vecchione A, Hassan C, Winn S, *et al*. Gastric epithelial cell proliferation in patients with liver cirrhosis. Dig Dis Sci 2001; 46:550–554 .
22. Celinski K, Konturek PC, Slomka M, Cichoz-Lach H, Gonciarz M, Bielanski W, *et al*. Altered basal and postprandial plasma melatonin, gastrin, ghrelin, leptin and insulin in patients with liver cirrhosis and portal hypertension without and with oral administration of melatonin or tryptophan. J Res 2009; 46:408-414.
23. Izbéki F,Kiss I,Wittmann T, Várkonyi T, Várkonyi TT, Légrády P, *et al.*Impaired accommodation of proximal stomach in patients with alcoholic liver cirrhosis. Scand J Gastroenterol 2002; 37: 1403-1410.
24. Nam YJ, Kim SJ, Shin WC, Lee JH, Choi WC, Kim KY, Han TH.. Gastric pH and *Helicobacter pylori* Infection in Patients with Liver Cirrhosis. Korean J Hepatol 2004; 10:216-222.
25. Kamalaporn P, Sobhonslidsuk A, Jatchavala J, Atisook K, Rattanasiri S, Pramoolsinsap Cl. Factors predisposing to peptic ulcer disease in asymptomatic cirrhotic patients. Aliment Pharmacol Ther 2005; 21:1459–1465.
26. Konturek SJ, Gonciarz M, Gonciarz Z, Bielanski W, Mazur W, Mularczyk A, *et al*. Progastrin and its products from patients with chronic viral hepatitis and liver cirrhosis. Scand J Gastroenterol 2003; 38:643-647.
27. Dijkstra G, Van GH, Jansen PL, Moshage Hl. Targeting nitric oxide in the gastrointestinal tract. Curr Opin Investig Drugs 2004; 5:529-536.

28. Berg A, Kechagias S, Sjöstrand SE, Ericson AC. Morphological Support for Paracrine Inhibition of Gastric Acid Secretion by Nitric Oxide in Humans. Scand J Gastroenterol 2001; 36: 1016-1021.
29. Holm M, Powell T, Casselbrant A, Johansson B, Fa¨ndriks L. Dynamic involvement of the inducible type of nitric oxide synthase in acid-induced duodenal mucosal alkaline secretion in the rat. Dig Dis Sci 2001; 46:1765–1771.
30. Nylander O, Ha¨llgren A, Sababi M. COX inhibition excites enteric nerves that affect motility, alkaline secretion, and permeability in rat duodenum. Am J Physiol Gastrointest Liver Physiol 2001; 281:1169–1178.
31. Hasebe K, Horie S, Komasaka M, Yano S, Watanabe K. Stimulatory effects of nitric oxide donors on gastric acid secretion in isolated mouse stomach. Eur J Pharmacol 2001; 420:159-164.
32. Hasebe K, Horie S, Noji T, Watanabe K, Yano S. Stimulatory effects of endogenous and exogenous nitric oxide on gastric acid secretion in anesthetized rats. Nitric Oxide 2005; 13:264-271.