Role of bile acids, prostaglandins and COX inhibitors in chronic esophagitis in a mouse model

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AIM: To develop a new experimental model of esophagitis that serves a complementary tool to clinical investigation in an insight into the mechanism of the damage to the esophagus mucosa by aggressive factors, and role of COX inhibitors in this process.

METHODS: The study was conducted in 56 male mice. Animals were divided into seven groups: (1) perfused with HCl, (2) perfused with HCl and physiologic concentration of pepsin (HCl/P), (3) perfused with similar HCl/P solution enriched with conjugated bile acids (glycho- and tauro-sodium salts) designated esophageal infusion catheter under the general anesthesia, (4) perfused as in group 2 treated with indometacin, (5) perfused as in group 2 treated with NS-398, (6) perfused as in group 3 treated with indometacin, and (7) perfused as in group 3 treated with NS-398. The esophagus was divided into 3 parts: upper, middle and lower. The PGE2 concentration was measured in all parts of esophagus using RIA method. Esophagus of sacrificed animals was macroscopically evaluated using a low power dissecting microscope (20x). Specimens, representing the most frequently seen changes were fixed, stained with H&E and assessed microscopically using the damage score, and inflammatory score.

RESULTS: The macroscopic changes were significantly severer in HCl/P than those in HCl animals (77%) and in HCl/P/BA group (43%). In HCl/P NS-398 group we noticed significantly less changes than those in not treated group (42%) and in analogical group treated with indometacin (45%). In HCl/P/BA INDO group we observed significantly severer changes than that in not treated group (52%). We noticed less changes in HCl/P NS-398 than that in group with indomethacine (46%). In HCl/P/BA NS-398 group we had less changes than that in indometacin group (34%). The microscopic changes observed in HCl/P/BA/INDO group were severer than that in not treated group (48%). Esophagitis index in HCl group was significantly lower than in HCl/P and also HCl/P/BA group (32% and 33%). In HCl/P/BA/INDO group the esophagitis surface was larger than that in not treated group (33%). In HCl/P group the surface of esophagus with ulceration was significantly larger (10-fold) than that in HCl/P/BA group. The PGE2 concentration in lower part of esophagus was also significantly higher in middle than those in HCl and HCl/P/BA groups. In upper part of esophagus the PGE2 concentration was significantly higher in HCl/P/BA group than that in group treated with indomethacine (46%). We also observed higher PGE2 concentration in middle part of esophagus in HCl/P/BA group than those in group treated with indometacin and in group treated with indometacin and NS-398 (by 52% and 43% respectively).

CONCLUSION: Pepsin is the pivotal factor in the development of chronic esophageal injury. Bile acids diminish chronic esophageal injury induced by HCl/P, indicating its potential negative impact on pepsin proteolytic potential, pivotal for mucosal injury in low pH. The role of selective COX inhibitors is still unclear, and needs more investigations. This novel chronic experimental esophagitis is an excellent model for further study on the role of cytokines in genetically modified animals.

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Key words: Bile acids; Prostaglandins; Oesophagitis mouse model

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INTRODUCTION

Primary gastroesophageal reflux disease (GERD) is an ac-
id-related disease in majority of patients. However, there is evidence that in some patients with GERD reflux of duodenal contents into the stomach and esophagus may be involved in the disease\cite{1,2}.

Chronic GERD may induced Barrett’s metaplasia\cite{3}. This clinical situation has increased risk for the development of esophageal adenocarcinoma and is considerate to be a premalignant condition\cite{4}. The complications in Barrett’s esophagus was accompanied with presents of duodenal juice in gastroesophageal refluxate (GER)\cite{5}. In the patients with esophagitis, Barrett’s esophagus strictures compared to patients with minimal injury the concentration of bile acids in refluxate was significantly higher\cite{6}. The concentration of bile was significantly higher in patients with early adenocarcinoma arising in Barrett’s esophagus, compared to GERD patients, esophagitis group and asymptomatic volunteers\cite{7}. Clinical trials have begun in order to assess the efficacy of selective COX-2 inhibitors to prevent the progression of Barrett’s esophagus to adenocarcinoma. Bile salts and acid are likely to early induce COX-2 in this sequence, although other factors, such as proinflammatory cytokines, inducible nitric oxide synthase and growth factors such as TGF-beta, are potential COX-2 inducers in the esophagus\cite{9}. In animal studies it has been shown that reflux of gastric contents with addition of duodenal juice into the esophagus may lead to esophageal adenocarcinoma\cite{10}. The carcinogenic effect of duodenal contents on gastric mucosa was clearly demonstrated\cite{11}.

Bile acid may induce mucosal injury by two mechanisms. The detergent properties of bile salts may destabilize membranes and increase permeability, disrupt cellular homeostasis and potentially result in cell death\cite{12}. Bile acids may also create cytotoxic effect through cellular absorption of bile salts, which is dependent upon the salt’s ionization\cite{13}.

COX-1 activity is constitutive in the rabbit esophageal mucosa, but both COX-2 and COX-1 activity are increased under the impact of acidified pepsin. Treatment with the COX-2 inhibitor DFU is associated with improvement of mucosal damage, which may have therapeutic implications\cite{14}.

PGE2 plays the important role in development of Barrett’s esophagus and adenocarcinoma of the esophagus. The concentration of PGE2 was significantly higher in high grade displasia cells and also in adenocarcinoma cells of esophagus\cite{15}.

Our new experimental model of chronic esophagitis seems to be very useful tool to determinate the role of HCl/P/BA, major components of duodenogastroesophageal reflux, and the role of COX inhibitors on pathological changes in mucosa of the esophagus during refluxate episodes.

**MATERIALS AND METHODS**

The study was conducted in 56 male mice (CD1 strain from Charles River) according to study protocol approved by Animal Research Committee at KUMC. Animals were divided into seven groups: (1) Animal perfused with HCl (100 mmol/l, pH1.1), (2) Animals perfused with HCl (100 mmol/l, pH1.1) and physiologic concentration of pepsin (0.5 mg/l of HCl) (HCl/P), (3) Animals perfused with similar HCl/P solution enriched with conjugated bile acids (glico- and tauro-sodium salts) designated esophageal infusion catheter under the general anesthesia, (4) Animals perfused as in group 2 treated with indometacin (5 mg/kg b.w.s.c.), (5) Animals perfused as in group 2 treated with NS-398 (10 mg/kg b.w.p.o), (6) Animals perfused as in group 3 treated with indometacin (5 mg/kg b.w.s.c.), and (7) Animals perfused as in group 3 treated with NS-398 (10 mg/kg b.w.p.o). The total perfusion time per day for each mouse was 1.5 h. At the end of experimental procedure the animals were sacrificed under prolonged metoxyflurane anesthesia, esophagus was removed, opened and evaluated microscopically after stained with Alcian blue (0.1%, pH 5.8), using a low power dissecting microscope (20×) with stage micrometer for measurement of the area of macroscopic damage. The macroscopic changes was evaluated based on macroscopic score: 0 - no changes; 1 - erosion - max 3, size - 3-6 mm; 2 - erosion - 6 and up, size - 6-9 mm; 3 - ulcer without perforation with small hemorrhagic areas; 4 - ulcer with perforation and large hemorrhagic areas.

Specimens, representing the most frequently seen changes were fixed, stained with hematoxylin and cosin, and assessed microscopically using the damage score\cite{16}: 1-normal esophagus, 2-submucosal edema or separation of epithelial layer, 3-focal areas of intramural hemorrhage or partial epithelial loss, 4 - large areas of hemorrhage or complete epithelial desquamation; and inflammatory score\cite{17}, 0 - no infiltration, 1-very mild infiltration, 2-mild infiltration, 3-moderate infiltration, 4-marked infiltration.

The concentration of PGE2 was measured in 1/3 upper, 1/3 middle and 1/3 lower parts of esophagus using RIA kit (Amersham, Arlington Heights, Illinois).

Statistical analysis was performed with S-Stat (Jandel Sci. Co).

**RESULTS**

The macroscopic score was significantly higher in animals perfused with HCl/P than those in groups with HCl/P/BA and with HCl (3.69±0.23 vs 2.58±0.25 and 3.69±0.23 vs 2.08±0.11, P<0.05). The macroscopic score was significantly lower in group HCl/P/NS-398 than that in not treated group (2.13±0.21 vs 3.69±0.23, P<0.05) and also lower than that in analogical group treated with indometacin. In HCl groups, the microscopic changes were less evident in groups with HCl and HCl/P/BA than that in HCl/P (2.63±0.38 vs 3.90±0.10 and 2.64±0.27 vs 3.90±0.10, P<0.05, respectively). The microscopic score was the same in HCl group and in HCl/P/BA group. The microscopic changes were significantly severer in group HCl/P/BA/INDO than that in not treated group (3.90±0.10 vs 2.64±0.27, P<0.05) and also than that in analogical group treated with NS-398 (3.90±0.10 vs 2.58±0.14, P<0.05). We noticed significantly higher score of microscopic changes in group HCl/P/INDO than that in group treated with NS-398 (3.92±0.07 vs 2.12±0.13, P<0.05).

Inflammation of esophagus in HCl group was significantly lower than that in HCl/P group (2.63±0.24 vs 3.90±0.10, P<0.05). The inflammation score in HCl/P/BA group was also lower than that in HCl/P (2.23±0.26 vs 3.90±0.10, P<0.05). Inflammation was less evident in HCl/P/NS-398 than those in not treated group and in group treated with indometacin (2.21±0.11 vs 3.90±0.10 and
2.21 ± 0.11 vs 3.86 ± 0.11 respectively, P < 0.05). Inflammation score was higher in HCl/P/BA/INDO than those in not treated group and in group treated with NS-398 (3.93 ± 0.03 vs 2.23 ± 0.26 and 3.93 ± 0.03 vs 2.42 ± 0.13 respectively, P < 0.05). Esophagitis index in HCl group was significantly lower than those in HCl/P and HCl/P/BA groups (15.50 ± 2.02 vs 23.00 ± 2.31 and 15.50 ± 2.02 vs 23.46 ± 3.85 % of all esophagus surface, P < 0.05). Surface area of esophagitis was significantly larger in HCl/P/BA/INDO group than that in not treated one (31.17 ± 2.45 vs 23.46 ± 3.85, P < 0.05) and also larger than that in group treated with NS-398 (31.17 ± 2.45 vs 21.23 ± 1.21, P < 0.05). In the HCl group of animals we did not observe any ulceration of the esophagus. In HCl/P group the surface of esophagus with ulceration was significantly larger than in HCl/P/BA (7.09 ± 2.17 vs 0.71 ± 0.49, P < 0.05). The surface of ulceration in esophagus was significantly larger in HCl/P/BA/INDO group than that in not treated group (1.35 ± 0.11 vs 0.71 ± 0.49, P < 0.05). All data are showed in Table 1.

In the HCl group the concentration of PGE2 in middle part of esophagus was significantly higher than in lower part (1.02 ± 0.11 vs 0.27 ± 0.09 pg/mg of protein, P < 0.05). We also observed higher concentration of PGE2 in the middle part of esophagus than that lower one in animals from HCl/P/BA groups (1.01 ± 0.13 vs 1.23 ± 0.13 mg, P < 0.05). In the HCl/P/BA group the concentration of PGE2 was significantly higher in the middle part of esophagus than that observed in the HCl/P (1.24 ± 0.13 mg of protein vs 0.87 ± 0.11 mg, P < 0.05). The concentration of PGE2 in upper part of esophagus in HCl/P/BA/INDO group was significantly lower than that in not treated group (553 ± 50 vs 807 ± 111 pg/mg of protein, P < 0.05). We noticed lower PGE2 concentration in middle part of esophagus in HCl/P/BA treated with indometacin and NS-398 than in not treated analogical group (614 ± 64 vs 1264 ± 134 and 733 ± 67 vs 1 264 ± 134 pg/mg of protein respectively, P < 0.05). All data are shown in Table 2.

**DISCUSSION**

In our current study we demonstrated the significantly increase of macroscopic damage score in esophageal mucosa in animals perfused with HCl/P when compared with HCl and HCl/P/BA group. In addition in group perfused with HCl/P the microscopic changes were significantly remarkable compared to that in HCl and HCl/P/BA perfused animals. Inflammation of esophagus in HCl/P group was evidently severer than that in HCl only perfused animals. Inflammation of the esophageal mucosa in group with perfusion mimicking duodenogastroesophageal reflux was significantly higher than in HCl and HCl/P/BA perfused groups. The total surface of esophagitis in HCl/P perfused animals was significantly larger than that in HCl perfused group of animals. We observed wider surface of esophagitis in group perfused with HCl/P/BA – mimicking duodenogastroesophageal reflux, than in HCl/P perfused animals but the differences was not significant. However, the surface of esophagus with esophagitis in group mimicking the duodenogastroesophageal reflux was significantly larger than that observed in HCl perfused group. In group of mice with perfusion of esophagus with HCl we did not find any ulceration. We demonstrated in group with HCl/P significantly larger surface of esophagus with ulcer (10-fold) than in HCl/P/BA group. In our study we also found the significant decrease of macroscopic damage in esophageal mucosa in group of animals perfused with HCl/P and treated with NS-398. Macroscopic score in this group was lower than that in analogical group treated...
with indomethacine. We also observed this phenomenon in microscopic and inflammatory scores. We noticed that animals perfused with HCl/P/BA and treated indomethacine had higher values of all macro- and microscopical scores than in not treated group. In addition, HCl/P/BA group of mice treated with NS-398 showed lower values of all macro-and microscopical scores than that observed in animals treated with indomethacine.

It was recently documented that in patients with reflux esophagitis the concentration of bile acids in refluxate is significantly higher than that in asymptomatic volunteers\(^3,13,16\).

Chronic GERD may induced Barrett’s metaplasia\(^3\). This clinical situation has increased risk for the development of esophageal adenocarcinoma and is considered to be a premalignant condition\(^9\). The concentration of bile was significantly higher in patients with early adenocarcinoma arising in Barrett’s esophagus, compared to GERD patients, esophagitis group and asymptomatic volunteers\(^9\).

In our previous clinical study we demonstrated that perfusion with acid, pepsin and bile acids, mimicking the duodenogastroesophageal reflux episodes increased the esophageal protective components secretion in asymptomatic volunteers, and less evidently in GERD patients.

There are some surgical experimental model of esophagitis, Barrett’s esophagus and also adenocarcinoma of esophagus\(^13,17\). In animal studies it has been shown that reflux of gastric contents with addition of duodenal juice into the esophagus may lead to esophageal adenocarcinoma\(^19\). The carcinogenetic effect of duodenal contents on gastric mucosa was clearly demonstrated\(^9\). The higher concentration of PGE2 in esophagus may be connected with deeper impact of bile acids on the esophagus wall, and induction of COX-2 in the esophagus muscle cells\(^9\). The role of COX-2 inhibitors in that phenomenon is still unclear and need more experiments.

Our new experimental model of esophagitis in mice mimicking the clinical scenario of gastroesophageal or duodenogastroesophageal reflux seems to be a useful tool to investigate some pathological problems in esophageal pathophysiology.

In conclusion, pepsin is the pivotal factor in the development of chronic esophageal injury. Bile acids diminish chronic esophageal injury induced by HCl/P, indicating its potential negative impact on pepsin proteolytic potential, pivotal for mucosal injury in low pH. The COX-2 inhibitors are much more active than not selective inhibitors in patients with esophageal mucosa injury, especially during duodenogastroesophageal reflux scenario. This novel chronic experimental esophagitis is an excellent model for further study on the role of cytokines in health and disease of the esophageal mucosa in genetically modified animals.

REFERENCES

1 Fiorucci S, Santucci L, Chiucciù S, Morelli A. Gastric acidity and gastroesophageal reflux patterns in patients with esophagitis. *Gastroenterology* 1992; 103: 855-861

2 Attwood SE, DeMeester TR, Bremner CG, Barlow AP, Hinder RA. Alkaline gastroesophageal reflux: implications in the development of complications in Barrett’s columnar-lined lower esophagus. *Surgery* 1989; 106: 764-70

3 Seabrook M, Holt S, Gilrane T. Barrett’s esophagus: observations on diagnosis and management. *South Med J* 1992; 85: 280-288

4 Spechler SJ, Goyal RK. Barrett’s esophagus. *N Engl J Med* 1986; 315: 362-371

5 Nehra D, Howell P, Williams CP, Pye JK, Beynon J. Toxic bile acids in gastro-oesophageal reflux disease: influence of gastric acidity. *Gut* 1999; 44: 598-602

6 Stein HJ, Kauer WK, Feusner H, Siewert JR. Bile reflux in benign and malignant Barrett’s esophagus: effect of medical acid suppression and nissen fundoplication. *J Gastrointest Surg* 1998; 2: 333-341

7 Piazuelo E, Jimenez P, Lanas A. COX-2 inhibition in esophagitis, Barrett’s esophagus and esophageal cancer. *Curr Pharm Des* 2003; 9: 2267-2280

8 Taylor PR, Mason RC, Filipe MJ, Vaja S, Hanley DC, Murphy GM, Dowling RH, McColl I. Gastric carcinogenesis in the rats induced by duodenogastric reflux without carcinogens: morphology, mucin histochemistry, polyamine metabolism and labelling index. *Gut* 1991; 32: 1447-1454

9 Stipa E, Stein HJ, Feusner H, Kraemer S, Siewert JR. Assessment of non-acid esophageal reflux: comparison between long-term reflux aspiration test and fiberoptic bilirubin monitoring. *Dis Esophagus* 1997; 10: 24-28

10 Vaezi MF, Richter JE. Role of acid and duodenogastroesophageal reflux in gastroesophageal reflux disease. *Gastroenterology* 1996; 111: 1192-1199

11 Lanas A, Jimenez P, Ferrandez A, Escartin A, Arenas J, Esteva F, Ortejo J. Selective COX-2 inhibition is associated with decreased mucosal damage induced by acid and pepsin in rabbit esophagitis. *Inflammation* 2003; 27: 21-29

12 Morgan G, Vainio H. Barrett’s oesophagus, oesophageal cancer and colon cancer: an explanation of the association and cancer chemopreventive potential of non-steroidal anti-inflammatory drugs. *Eur J Cancer Prev* 1998; 7: 195-199

13 Tay HP, Chaparala RC, Harmon JW, Huesken J, Saini N, Hakki FZ, Schweitzer EJ. Bismuth subsalicylate reduces peptic injury of the oesophagus in rabbits. *Gut* 1990; 31: 11-16

14 Trevellick MA, Clayton NM, Strong P, Harman IW. Do infiltrating neutrophils contribute to the pathogenesis of indomethacin induced ulceration of the rat gastric antrum? *Gut* 1993; 34: 156-160

15 Gotley DC, Morgan AP, Cooper MJ. Bile acid concentration in the refluxate of patients with reflux esophagitis. *Br J Surg* 1988; 7: 587-590

16 Nehra D, Howell P, Pye JK, Beynon J. Assessment of combined bile acid and pH profiles using an automated sampling device in gastro-oesophageal reflux disease. *Br J Surg* 1998; 85: 134-137

17 Goldstein SR, Yang GY, Curtis SK, Reuhl KR, Liu BC, Mirvish SS, Newmark HL, Yang CS. Development of esophageal metaplasia and adenocarcinoma in a rat surgical model without the use of a carcinogen. *Carcinogenesis* 1997; 18: 2265-2270

18 Wetscher G, Perdikis G, Ketchmar D. Esophagitis in Sprague-Dawley rats is mediated by free radicals. *Dig Dis Sci* 1995 ; 40: 1297-1305

19 Attwood SE, Smyrk TC, DeMeester TR, Mirvish SS, Stein HJ, Hinder RA. Duodenoesophageal reflux and the development of esophageal adenocarcinoma in rats. *Surgery* 1992; 111: 503-510