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Role of Bile Acids and Prostaglandins in Chronic Esophagitis in not Surgical Mouse Model*

Rola soli kwasów żółciowych i prostaglandyn w eksperymentalnym zapaleniu przełyku

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Abstract

Objectives. Development of a new experimental model of esophagitis that serves complementary tool to clinical investigative in an insight into the mechanism of the damage to the esophagus mucosa by acid pepsin and bile components, mimicking the natural duodenogastroesophageal reflux scenario.

Material and Methods. The study was conducted in 24 male animals (CD1 strain from Charles River). The animals were divided into three groups: 1) animal perfused with HCl (100 mM, pH 1.1), 2) animals perfused with HCl (100 mM, pH 1.1) 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 (glycho- and tauro-sodium salts) designated esophageal infusion catheter under the general anesthesia. The esophagus was divided in 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 (20×) with stage micrometer for measurement of the area of macroscopic damage. Specimens, representing the most frequently seen changes were fixed, stained with HE and assessed microscopically using the damage score (from 0 to 4), and inflammatory score (from 0 to 4).

Results. The mean time (days) of total experiment in that group of mice perfused with HCl was statistically significant longer than in HCl/P group and HCl/P/BA group (100% and 124%, $p < 0.05$, respectively). The macroscopic changes were significantly bigger in HCl/P than in HCl animals (by 77%, $p < 0.05$) and also than in HCl/P/BA group (by 43%, $p < 0.05$). The microscopic changes were less evident in group with HCl than in HCl/P (32.5%, $p < 0.05$). Inflammation of esophagus in HCl group was significantly lower than in HCl/P group (32.5%, $p < 0.05$). The inflammation score in HCl/P/BA group was also lower than in HCl/P (57%, $p < 0.05$). Esophagitis index in HCl group was significantly lower than in HCl/P and also HCl/P/BA group (32% and 33%, $p < 0.05$). In the HCl group of animals the authors did not observe any ulceration of the esophagus. In HCl/P group the surface of esophagus with ulceration was significantly bigger (10-fold) than in HCl/P/BA group ($p < 0.05$). The PGE2 concentration was significantly higher in HCl/P group than in HCl/P/BA group ($p < 0.05$). The PGE2 concentration was also significantly higher in middle than in lower part of esophagus in HCl and HCl/P/BA groups.

Conclusions. Pepsin is the pivotal factor in the development of chronic esophageal injury. Bile acids diminish chronic esophageal injury induced by HCl/P indicating its potentially negative impact on pepsin proteolytic potential, pivotal for mucosal injury in low pH. This novel chronic experimental esophagitis is an excellent model for future study on the role of cytokines in health and disease of the esophageal mucosa in genetically modified animals (Adv Clin Exp Med 2005, 14, 4, 657–661).

Key words: chronic esophagitis, bile acids, prostaglandins, experimental model, mice.

* This work was supported by State Committee for Scientific Research (KBN).

Streszczenie

Cel pracy. Opracowanie nowego modelu zapalenia przełyku, który jest przydatnym narzędziem do badań nad mechanizmami uszkodzenia błony śluzowej przełyku przez kwas solny i sole kwasów żółciowych. Przedstawiony model przewlekłego zapalenia przełyku umożliwia badanie zmian zachodzących w warunkach klinicznych u pacjentów z chorobą refluksową.

Materiał i metody. Eksperyment przeprowadzono na 24 myszach, samcach rasy Suiss. Zwierzęta podzielono na 3 grupy: 1) myszy poddane perfuzji przełyku kwasem solnym HCl (100 mM; pH 1,1), 2) myszy poddane perfuzji przełyku mieszaniną HCl (100 mM, pH 1,1) i pepsyny (0,5 mg/l HCl) – HCl/P, 3) zwierzęta poddane perfuzji przełyku jak w grupie 2. z dodatkiem soli kwasów żółciowych HCl/P/BA. Perfuzję przeprowadzono z użyciem specjalnego cewnika w znieczuleniu ogólnym. Przełyki myszy po zakończeniu eksperymentu zostały pobrane i podzielone na 3 części: górną, środkową i dolną. Stężenie PGE2 określono w każdej z trzech części przełyku metodą RIA. Przełyki oceniono makroskopowo (powiększenie 20×) z jednoczesnym mikrometrycznym pomiarem powierzchni zmian. Wykonano ocenę mikroskopową zmian w obrębie przełyku (metodą HE) i określono współczynnik uszkodzenia (0–4) i współczynnik nacieku zapalnego (0–4).

Wyniki. Średni czas trwania perfuzji w grupie HCl był znacznie dłuższy niż w grupie HCl/P i HCl/P/BA (odpowiednio 100% i 124%; $p < 0,05$). Zmiany makroskopowe były znacznie większe w grupie HCl/P niż w HCl (o 77%; $p < 0,05$), oraz niż w HCl/P/BA (o 43%; $p < 0,05$). Zmiany mikroskopowe były mniej nasilone w grupie HCl niż w HCl/P (o 32,5%; $p < 0,05$). Naciek zapalny był znacznie mniejszy w grupie HCl niż w grupie HCl/P (o 32,5%; $p < 0,05$) a mniejszy w grupie HCl/P/BA niż w grupie HCl/P (o 57%; $p < 0,05$). Indeks zapalenia przełyku w grupie HCl był istotnie mniejszy niż w HCl/P i HCl/P/BA (odpowiednio o 32 i 33%; $p < 0,05$). W grupie HCl nie obserwowano owrzodzeń w obrębie błony śluzowej przełyku. Powierzchnia owrzodzeń przełyku w grupie HCl/P była 10 razy większa niż w HCl/P/BA ($p < 0,05$). Stężenie PGE2 było wyższe w grupie HCl/P niż w HCl/P/BA ($p < 0,05$).

Wnioski. Pepsyna jest głównym czynnikiem uszkadzającym w przewlekłym zapaleniu przełyku. Sole kwasów żółciowych zmniejszają uszkadzający wpływ pepsyny na błonę śluzową przełyku przez zmniejszenie zdolności proteolitycznych pepsyny w małym pH. Zaprezentowany nowy model zapalenia przełyku jest doskonałym narzędziem do badania wpływu cytokin na uszkodzenie błony śluzowej przełyku z wykorzystaniem w eksperymencie genetycznie modyfikowanych myszy (*Adv Clin Exp Med* 2005, 14, 4, 657–661).

Słowa kluczowe: przewlekłe zapalenie przełyku, sole kwasów żółciowych, prostaglandyny, model eksperymentalny, myszy.

Primary gastroesophageal reflux disease (GERD) is an acid-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 [1, 2].

Chronic GERD may induce Barrett's metaplasia [3]. This clinical situation has increased risk for the development of esophageal adenocarcinoma and is considered to be a premalignant condition [4]. The complications in Barrett's esophagus were accompanied with presence of duodenal juice in gastroesophageal refluxate (GER) [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 [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 [7].

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 [8]. The carcinogenetic effect of duodenal contents on gastric mucosa was clearly demonstrated [9].

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 [10]. Bile acids may also create cytotoxic effect through cellular absorption of bile salts, which is dependent upon ionization of the salt [11]. PGE2 plays important role in development of Barrett's esophagus and adenocarcinoma of the esophagus. The concentration of PGE2 was significantly higher in high grade dysplasia cells and also in adenocarcinoma cells of esophagus [12].

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

Material and Methods

The study was conducted in 24 male mice (CD1 strain from Charles River) according to study protocol approved by Animal Research Committee at KUMC. The animals were divided into three groups: 1) animals perfused with HCl (100 mM, pH 1.1) and physiologic concentration of pepsin

(0.5 mg/l of HCl) – HCl/P), 2) animals perfused with similar HCl/P solution enriched with conjugated bile acids (glycho- and tauro-sodium salts from Sigma Chem Co) in a composition mimicking the human bile using own specially designated esophageal infusion catheter under the general anesthesia. The total perfusion time per day for each mouse was 1.5 hour.

At the end of experimental procedure the animals were sacrificed, under prolonged metoxyflurane anesthesia esophagus removed, opened and evaluated microscopically 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 were evaluated basing on macroscopic score: 0 – no changes; 1 – erosiones: max 3, size: 3–6 mm; 2 – erosiones: 6 and up, size: 6–9 mm; 3 – ulcer without perforation with small haemorrhagic areas; 4 – ulcer with perforation and large haemorrhagic areas.

Specimens, representing the most frequently seen changes were fixed, stained with HE and assessed microscopically using the damage score [13]: 1 – normal esophagus, 2 – submucosal oedema or separation of epithelial layer, 3 – focal areas of intramural haemorrhage or partial epithelial loss, 4 – large areas of haemorrhage or complete epithelial desquamation; and inflammatory score [14]: 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 part of esophagus using RIA kit (Amersham, Arlington Heights, Illinois).

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

Results

The mean time of total experiment in the group of mice perfused with HCl was statistically significant longer than in HCl/P group and HCl/P/BA group (13 ± 0.85 vs. 6.50 ± 0.43 , 13 ± 0.85 vs. 5.79 ± 0.76 days, $p < 0.05$, respectively). The macroscopic score was significantly higher in animals perfused with HCl/P than in group with HCl/P/BA and also higher than in mice perfused 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 microscopic changes were less evident in group with HCl and HCl/P/BA than 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. Inflammation of esophagus in HCl group was significantly lower than 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 in HCl/P (2.23 ± 0.26 vs. 3.90 ± 0.10 , $p < 0.05$). Esophagitis index in HCl group was significantly lower than in HCl/P and also HCl/P/BA group (15.50 ± 2.02 vs. 23.00 ± 2.31 and 15.50 ± 2.02 vs. 23.46 ± 3.85 per cent of all esophagus surface, $p < 0.05$). In the HCl group of animals the authors did not observe any ulceration of the esophagus. In HCl/P group the surface of esophagus with ulceration was significantly bigger than in HCl/P/BA group (7.09 ± 2.17 vs. 0.71 ± 0.49 mm², $p < 0.05$). All data in Table 1.

In the HCl group the concentration of PGE2 in middle part of esophagus was significantly higher than in lower part (1027 ± 166 pg/mg of protein vs. 378 ± 69 pg/mg of protein, $p < 0.05$). The authors also observed the higher concentration of PGE2 in the middle part of esophagus than in lower one in animals from HCl/P/BA groups ($1264 \pm$

Table 1. Macroscopic and microscopic changes in mice esophageal mucosa (mean \pm SE)

Tabela 1. Makroskopowe i mikroskopowe zmiany błony śluzowej przełyku u myszy (średnia \pm SE)

Model (Model)	Mean time of perfusion (Średni czas perfuzji) days – doby	Grades of macroscopic changes (Stopień zmian makroskopowych)	Grades of microscopic changes (Stopień zmian mikroskopowych)	Grades of inflammation (Stopień zapalenia)	Surface of esophagitis – % of all esophagus (Powierzchnia zapalenia przełyku – % całego przełyku)	Ulcer of esophagus (Wrzody przełyku) mm ²
HCl	13.25 ± 0.85^{AB}	2.08 ± 0.11	2.63 ± 0.38	2.63 ± 0.24	15.50 ± 2.02	0 ± 0.0
HCl/P	6.50 ± 0.43	3.69 ± 0.23^A	3.90 ± 0.10^A	3.90 ± 0.10^A	23.00 ± 2.31^A	7.09 ± 2.17^A
HCl/P/BA	5.79 ± 0.76	2.58 ± 0.25^B	2.64 ± 0.27^B	2.23 ± 0.26^B	23.46 ± 3.85^A	0.71 ± 0.49^{AB}

^A $p < 0.05$ vs. HCl.

^B $p < 0.05$ vs. HCl/P.

HCl/P/BA = HCl + pepsin + bile acids.

HCl/P/BA = HCl + pepsyna + sole kwasów żółciowych.

Table 2. Concentration of PGE2 in mouse esophagus (mean \pm SE pg/mg of protein)**Tabela 2.** Stężenie PGE2 w mysim przełyku (średnia \pm SE pg/mg białka)

Model (Model)	1/3 upper part (górną część)	1/3 middle part (środkowa część)	1/3 lower part (dolną część)
HCl	801 \pm 103	1027 \pm 166 ^A	378 \pm 69 ^A
HCl/P	674 \pm 107	766 \pm 95 ^B	405 \pm 39
HCl/P/BA	807 \pm 111	1264 \pm 134 ^{A, B}	332 \pm 59 ^A

^A $p < 0.05$ middle vs. lower.^B $p < 0.05$ HCl/P vs. HCl/P/BA.^A $p < 0.05$ środkowa część vs dolna część.^B $p < 0.05$ HCl/P vs HCl/P/BA.

± 134 pg/mg of protein vs. 332 ± 59 pg/mg of protein, $p < 0.05$). In the HCl/P/BA group the concentration of PGE2 was significantly higher in the middle part of esophagus than observed in the HCl/P (1264 ± 134 pg/mg of protein vs. 766 ± 95 pg/mg of protein, $p < 0.05$). All data in Table 2.

Discussion

In the current study the authors demonstrated the significantly increased macroscopic damage score in esophageal mucosa in animals perfused with HCl/P when compared with HCl and also HCl/P/BA group. In addition in group perfused with HCl/P the microscopic changes were significantly bigger than in HCl and HCl/P/BA perfused animals. Inflammation of esophagus in HCl/P group was evidently more severe than 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 group. The total surface of esophagitis in HCl/P perfused animals was significantly bigger than in HCl perfused group of animals. The authors observed bigger surface of esophagitis in group perfused with HCl/P/BA – mimicking duodenogastroesophageal reflux, than in HCl/P perfused animals but the differences were not significant. However, the surface of esophagus with esophagitis in group mimicking the duodenogastroesophageal reflux was significantly bigger than observed in HCl perfused group. In group of mice with perfusion of esophagus

with HCl the authors did not find any ulceration. The authors demonstrated in group with HCl/P significantly bigger surface of esophagus with ulcer (10-fold) than in HCl/P/BA group.

It is was recently documented that in patients with reflux esophagitis the concentration of bile acids in refluxate is significantly higher than in asymptomatic volunteers [15, 16].

Chronic GERD may induce Barrett's metaplasia [17]. This clinical situation has increased risk for the development of esophageal adenocarcinoma and is considered to be a premalignant condition [18]. 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 [19].

In the previous clinical study the authors 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 [20]. There are some surgical experimental models of esophagitis, Barrett's esophagus and also adenocarcinoma of esophagus [21, 22].

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 [23]. The carcinogenetic effect of duodenal contents on gastric mucosa was clearly demonstrated [24]. 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 [25]. The role of COX-2 inhibitors in that phenomenon is still unclear and needs more experiments.

Presented new not surgical experimental model of esophagitis in mice mimicking the clinical scenario of gastroesophageal or duodenogastroesophageal reflux seems to be a useful tool to develop some pathological problems in esophageal pathophysiology.

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. This novel chronic experimental esophagitis is an excellent model for future study on the role of cytokines in health and disease of the esophageal mucosa in genetically modified animals.

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Received: 24.11.2004
Revised: 14.12.2004
Accepted: 27.12. 2004

Praca wpłynęła do Redakcji: 24.11.2004 r.
Po recenzji: 14.12.2004 r.
Zaakceptowano do druku: 27.12. 2004 r.