Immunohistochemical analysis of cell kinetics of the gastric- and esophageal epithelium in response to various stimuli

PhD thesis

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Introduction

Maintaining cell turnover is a key feature in organs with high metabolism such as gastric mucosa and esophageal epithelium. Increased cell turnover may lead to tumor development while the suppressed state results in mucosal damage and ulcer formation.

Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most widely used groups of drugs. Although these compounds represent a very effective class of medicines, their use is associated with a broad spectrum of untoward reactions, among the gastrointestinal especially gastroduodenal complications are the most significant.

NSAIDs have the common property of treating fever, pain, and inflammation by inhibiting synthesis of prostaglandins. NSAIDs bind reversibly or irreversibly to cyclooxygenase (COX) enzymes. COX-1-derived prostaglandins are responsible for mucosal defense and cytoprotection in the gastrointestinal tract (GI), while COX-2-derived prostaglandins mediate inflammation, pain and fever. Most NSAIDs are nonselective, blocking both, COX-1 and COX-2 isoenzymes. Deleterious effects of nonselective NSAIDs (ns-NSAIDs) on gastroprotection result from their inhibition of both isoenzymes, however inhibition of COX-1 is still believed to be of pivotal importance.

Proton pump inhibitor (PPI) co-therapy is considered as the best strategy in preventing gastrointestinal complications during NSAID treatment but there is limited information available on their effect on gastric mucosal cell kinetics.

PPI therapy causes profound and continuous hypochlorhydria by selective inhibition of the proton pump (H^+/K^+-ATPase) in gastric parietal cells. It has been reported that long term omeprazole treatment may reversibly increase epidermal cell proliferation and suppress its differentiation; however the results from different studies are controversial.

Barrett's esophagus (BE) is a condition in which the normal stratified squamous epithelium is replaced by metaplastic columnar epithelium that predisposes to the development of esophageal adenocarcinoma. Neoplastic progression in BE occurs by a multistep process associated with early molecular and morphological changes. Diagnosis of Barrett’s adenocarcinoma is usually made late, and consequently, is associated with poor prognosis. Earlier detection of cancer and/or characterization of dysplasia are beneficial in early identification of patients at higher risk for adenocarcinoma. Evaluation of tissue biomarkers has been proven useful for identifying dysplasia and estimation of malignant progression in BE.

Secretory leukocyte protease inhibitor (SLPI) represents a multifunctional protein of the gastrointestinal mucosa exerting antimicrobial and anti-inflammatory effects. SLPI expression is generally induced during inflammation; however, *Helicobacter pylori* (*H. pylori)*-mediated gastritis is associated with significantly decreased antral SLPI levels.

Aims

NSAID study
To evaluate changes in apoptosis and proliferation and expression of p53 and epithelial growth factor receptor (EGFR) of the gastric mucosa in chronic ns-NSAID- or selective COX-2 users with or without PPI co-therapy

PPI study
To analyze the effect of PPI therapy on cell proliferation, apoptosis and p53- and EGFR expression of the gastric epithelium

Barrett study
To evaluate cell proliferation and p53 expression and expression of glutathione S-transferase (GST) and matrix metalloproteinase-9 (MMP-9) in the development and progression of esophageal adenocarcinoma including reflux esophagitis, BE, BE with concomitant esophagitis, dysplasia and adenocarcinoma and estimate the correlation of expression of these markers in the whole sequence of malignant transformation of the esophagus

SLPI study
To analyze SLPI expression of the gastric mucosa in patients with chronic gastritis caused by different etiologies

Materials and Methods

NSAID study
Gastric biopsies of the antrum were taken from 10-10 patients on chronic ns-NSAID or selective COX-2 treatment prior to and after six months of PPI co-therapy, and from 10 healthy controls without any treatment. Proliferating cell nuclear antigen (PCNA), apoptosis, EGFR- and p53 expression were measured by immunohistochemistry.

PPI study

Gastric biopsies of the antrum were taken from patients with reflux esophagitis prior to and after six months of omeprazole (n=14) or esomeprazole (n=12) therapy. Cell proliferation, apoptosis, EGFR- and p53 expression were measured by immunohistochemical techniques.

Barrett study
Cell proliferation, p53-, GST- and MMP-9 expression were analyzed by immunohistochemistry in 51 paraffin-embedded tissue samples including patients with reflux esophagitis (n=7), BE (n=14), BE with concomitant esophagitis (n=8), Barrett's dysplasia (n=7), esophageal adenocarcinoma (n=8) and a control group without any histological changes (n=7).

SLPI study
SLPI expression was retrospectively analyzed by immunohistochemistry in 85 paraffin-embedded samples including *H. pylori*-associated chronic active gastritis (n=13), NSAID-induced gastritis (n=18), autoimmune gastritis (n=11), lymphocytic gastritis (n=26) and a control group without histological changes (n=17).

The intensity of the immunohistochemical staining was semiquantitatively analyzed using a counting method or immunoreactivity score. Statistical analysis with one-way ANOVA, LSD test and correlation analysis were performed. Values were expressed as mean ± SD and p value <0.05 was considered statistically significant.

Results

NSAID study
While p53 expression is increased and EGFR expression decreased, there is a trend towards increase of cell proliferation and apoptosis in the gastric mucosa after chronic ns-NSAID treatment but the difference does not reach statistical significance. Chronic administration of selective COX-2 inhibitors is associated with increased cell proliferation and decreased EGFR expression of the gastric epithelium but is not accompanied by increased p53 expression, while the apoptosis shows a trend towards increase. PPI co-therapy normalizes the disturbed cell kinetics irrespective of NSAID treatment used.

PPI study
Six month PPI treatment alone does not significantly increase gastric epithelial cell proliferation and EGFR expression and has no effect on

apoptosis or p53 expression. There is no difference between the effects of long term omeprazole or esomeprazole therapy on gastric epithelial cell kinetics. Although there is a trend towards increase in cell proliferation and EGFR expression of the gastric epithelium in both, omeprazole and esomeprazole-treated groups, the difference is not statistically significant. We found alterations only in the localization of immunohistochemical staining density prior to and after PPI therapy, regardless of the type of PPI.

Barrett study

While cell proliferation is significantly lower in the control group compared with all other groups, there is no increase in p53 expression of esophageal tissues that are negative for dysplasia including BE and reflux esophagitis. BE tissues that are positive for dysplasia reveal a significantly higher cell proliferation and p53 expression levels compared to BE, reflux esophagitis or BE with concomitant esophagitis. Both, cell proliferation and p53 expression are significantly higher in adenocarcinoma compared to BE or dysplasia. Interestingly, while just BE with concomitant esophagitis shows significantly higher p53 expression levels than BE alone, reflux esophagitis or the control group, both, BE with concomitant esophagitis and reflux esophagitis reveal significantly higher cell proliferation compared to BE alone. Alterations of cell proliferation and p53 expression show a strong correlation (r=0.91).

GST expression is significantly higher while MMP-9 expression is significantly lower in the control group compared to BE and the other groups. No major changes were found between BE, reflux esophagitis and BE with concomitant esophagitis. BE tissues that are positive for dysplasia and adenocarcinoma reveal a significant lower expression of GST and higher levels of MMP-9 compared to all other groups. Adenocarcinoma shows almost no expression of GST and significantly higher levels of MMP-9 than Barrett's dysplasia. Alterations of GST and MMP-9 are inversely correlated (r=-0.82).

SLPI study

In comparison with the control group, the SLPI expression of antral mucosa in *H. pylori*-mediated and lymphocytic gastritis is significantly lower, whereas epithelial SLPI expression is not affected in NSAID-induced and autoimmune gastritis either in the antrum or corpus, respectively. Both the *H. pylori*-associated and lymphocytic gastritis reveal a significantly lower expression of SLPI in infiltrating immune cells, whereas immune cells infiltrating the corpus in autoimmune gastritis show higher SLPI levels than the immune cells of other groups.

Conclusions

NSAID study

- p53 expression is increased and EGFR expression decreased in the gastric mucosa after chronic ns-NSAID treatment
- Chronic administration of selective COX-2 inhibitors is associated with increased cell proliferation and decreased EGFR expression of the gastric epithelium
- PPI co-therapy normalizes the disturbed cell kinetics irrespective of NSAID treatment used
- The prevalence of NSAID gastropathy is most likely not due to an effect on gastric cell turnover

PPI study

- There is no difference between the effects of long term omeprazole or esomeprazole therapy on gastric epithelial cell kinetics
- Six-month PPI treatment is not associated with cell turnover abnormalities
- Some alterations are only in the localization of immunohistochemical staining density of PCNA and EGFR prior to and after PPI therapy
- There is no risk for progression of hyperplasia to dysplasia in patients during PPI administration

Barrett study

- Overexpression of p53 is typical in the malignant transformation of BE and increases with histological progression
- Cell proliferation of Barrett's epithelium increases with progressive grades of dysplasia and is linearly correlated with p53 expression
- The simultaneous activation of cell proliferation and p53 expression strongly suggest their association with esophageal tumor genesis and their specific role in the biology of esophageal adenocarcinoma
- GST is downregulated, while MMP-9 is upregulated in reflux esophagitis-BE-dysplasia-adenocarcinoma sequence of the esophagus
- The loss of GST and gain on MMP-9 may indicate their specific role in the stepwise sequence and progression to carcinoma in BE
- Quantification of these biological markers in BE might be useful to identify patients at higher risk for progression to adenocarcinoma

SLPI study

- In *H. pylori*-associated and lymphocytic gastritis the SLPI staining of the mucosa is strongly reduced and frequently almost not detectable
- Epithelial SLPI expression is not affected in NSAID-induced or autoimmune gastritis
- SLPI downregulation is specifically linked to *H. pylori* infection and does not just represent a general phenomenon of gastric inflammation

Publications associated with the thesis

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