Abstract no.: P3.5

DIFFERENTIAL EXPRESSION OF HUMAN BETA DEFENSIN- 2 AND -3 IN GASTRIC MUCOSA OF *HELICOBACTER PYLORI*-INFECTED INDIVIDUALS

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Background: Antimicrobial peptides are keyplayers of initial innate immune responses to human pathogens. Two major representatives, the human beta defensin 2 and 3 (hBD2, hBD3) are both known to be induced by *Helicobacter pylori*. Previously, it was demonstrated in vitro that *H. pylori* actively abrogates hBD3 expression during prolonged infections. Here we comprehensively assessed hBD2 and hBD3 expression ex vivo in the gastric mucosa of healthy individuals.
Materials and Methods: Twenty volunteers (*H. pylori* positive and *H. pylori* negative: n = 10) were enrolled. *H. pylori* positive subjects underwent eradication therapy and repeated the protocol. Expression of both defensins were assessed by quantitative RT-PCR and ELISA, and correlated with histopathological degree of gastritis.
Results: hBD2 and hBD3 were found to be ubiquitously expressed in all three groups. In general, hBD2 levels were elevated in relation to *H. pylori* infection (up to 40-fold). This upregulation correlated with degree of gastritis in corpus and antrum. In contrast, hBD3 mRNA amounts were significantly decreased, while corresponding protein levels remained unchanged. Eradication therapy led to normalization of mucosal hBD2 expression, while hBD3 expression demonstrated high interindividual variations among individuals.
Conclusions: Both defensins are ubiquitously but differentially expressed in gastric mucosa in relation to *H. pylori* infection. Ex vivo data support previous in vitro findings that *H. pylori* infection is associated with reduced hBD3 expression in chronic active gastritis.

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BLOOD AND LYMPHATIC MICROVESSELS DENSITY IN GASTRIC MUCOSA OF DYSPEPTIC PATIENTS

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Introduction: Published data on the role of *H. pylori* in angiogenesis are contradictory, while its role in lymphangiogenesis has not been investigated.
Aims: To investigate the density of lymphatic and blood vessels, together with *H. pylori* infection, in biopsies of gastric body and antrum of dyspeptic patients.
Methods: Biopsies from patients subjected to gastroscopy according to clinical criteria were studied by immunohistochemistry. Exclusion criteria were: previous eradication therapy, ulcer disease, regular non-steroidal anti-inflammatory use, and proton pump inhibitor or antibiotic treatment 15 and 30 days prior to recruitment respectively. Microvessel density was determined with CD34 and D2.40 monoclonal antibodies (DAKO, Glostrup, Denmark), which are markers of blood and lymphatic vasculature respectively. Quantification was performed by direct counting of microvessels in four fields at 40× magnification. *H. pylori* infection was assessed by ^{13}C-urea breath test and/or histopathological diagnosis.
Results: Twenty-three biopsies (13 antral) from 13 patients were studied. Their median age was 62 years, 46% males, 73% gastritis and/or atrophy and 24% *H. pylori* positive. Higher blood microvessels densitiy was associated to *H. pylori* infection ($p = .03$) (Table 1). Microvessels count was slightly elevated, without statistical signification, among biopsies with histological diagnosis of gastritis. Further associations with inflammatory activity or location were not found. Lymphangiogenesis was not related with any of the studied variables.
Conclusions: (1) *H. pylori* infection was associated with increased gastric angiogenesis (2) Lymphangiogenesis was not related with the studied clinicopathological features.

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COX-2 INHIBITION WITH NUTRACEUTICALS: A NEW THERAPEUTIC APPROACH AGAINST *HELICOBACTER PYLORI* INFECTION?

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Accumulated evidence in humans and animals shows that *H. pylori* up-regulate the expression of cyclooxygenase (COX)-2 both at mRNA and protein levels which might be the one of the mechanisms leading to several gastric diseases.
Aim: To study the expression of COX-2 on mice gastric mucosa during long-term treatment with two nutraceuticals: curcumin and synbiotic 2000® on *H. pylori* experimental chronic infection.
Materials and Methods: We infected 45 C57BL/6 mice with SS1 – *H. pylori* strain. After infection confirmation by ^{13}C-urea breath test mice where then treated with either PBS, curcumin (10 mg/mouse) or Synbiotic 2000® (50 mg/mouse), three times per week. Five mice from each treatment group were euthanized at week 6, 18 and 27. Gastric samples were removed for COX-2 immunohistochemistry analysis.
Results: All the 45 mice were Hp positive by ^{13}C-urea breath test and immunohistochemistry. In the PBS group the production of COX-2 was significantly up-regulated at week 6 (area of positive immunostaining 393–544 × 10^3 pixels), 18 (area of positive immunostaining 242–614 × 10^3 pixels) and 27 week (area of positive immunostaining 129–175 × 10^3 pixels). The treatment with either curcumin or synbiotic significantly decreased the expression of COX-2 at all time points.
Conclusions: These results suggest the therapeutic usefulness of both nutraceuticals on COX-2 inhibition during chronic experimental mice *H. pylori* infection. The supplementation of diet in humans with curcumin or Synbiotic 2000® may be a novel therapeutic approach against gastric inflammation induced by Hp infection.

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PRODUCTION OF ANTI-*H. PYLORI* IMMUNOGLOBULIN Y (IGY) IN THE CHICKEN EGG YOLK

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Introduction: Immunizing chickens with certain antigens and collecting the related antibodies have been proposed as a useful method for production of edible immunoglobulins (Igs). Oral IgY could have applications in the control of gastric infections such as those caused by *Escherichia coli* and *Salmonella enterica*. In this study immunization of chicken and production of anti-*H. pylori* IgY in egg yolk was studied
Methods: A suspension of one heat-killed and PCR-confirmed *H. pylori* isolate mixed with freund's adjuvant was intramuscularly injected to two 5 months-old chickens, once a week for three consecutive weeks. Ten days after the last immunization, eggs were collected and total antibody was purified from egg yolk. The presence of anti-*H. pylori* IgY was assessed using dot blotting method on Polyvinylidene fluoride membrane. PCR-confirmed *E. coli* and *Salmonella entrica* were used to eliminate the possibility of cross-reaction.
Results: Specific binding of extracted antibodies to *H. pylori*-specific antigens was observed as colored spots on the membrane, indicating the presence of anti-*H. pylori* ploycolonal antibodies in the chicken eggs. Colorimetric reaction of antibodies with *E. coli* and *S. entrica*-specific antigens were not observed.
Discussion: Immunization of chickens for the production of antibodies has several advantages such as no need for blood sampling from animals and production of a large amount of antibodies in egg yolk. Furthermore, egg yolk containing anti-*H. pylori* antibody administered orally could provide a novel and effective approach to prevent *H. pylori* infection.

