UNIVERSITY OF MEDICINE AND PHARMACY OF CRAIOVA

STRUCTURAL AND ULTRASTRUCTURAL CHANGES OF GASTRIC MUCOSA INDUCED BY HELICOBACTER PYLORI INFECTION IN VARIOUS GASTRIC DISEASES

Summary

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INTRODUCTION

In 2005, Robin Warren and Barry Marshall have received the Nobel Prize in Physiology or Medicine for their discovery of the bacterium Helicobacter pylori and its role in gastritis and peptic ulcer disease. H. pylori infection, in his interaction with the host, is capable to adapt producing new genotypes through mutations and DNA rearrangements. High genetic variability of a strain to another, such as vacA and cagA, not only affects the body's ability to colonize and cause disease but also affects inflammation and gastric secretion.

My doctoral thesis proposes details and deciphering the pathogenic mechanisms of H. pylori infection:

- Highlight structural and ultrastructural changes of gastric mucosa induced by H.pylori infection;
- Means by which H.pylori interacts with gastric mucosa;
- Host body's response to H.pylori infection;
- Interaction of bacteria with gastric movements;
- Histochemical and immunohistochemical characterization of sequence events (atrophic gastritis, metaplasia, dysplasia, gastric cancer) which cause increased gastric disorders in patients infected with H. Pylori
Keywords: H. Pylori infection, Host body's response, structural and ultrastructural changes of gastric mucosa, gastric carcinogenesis

I. STATE OF KNOWLEDGE

Chapter 1. General considerations about H. Pylori infection: exhibits a brief history about H. pylori infection, a morphological and functional characterization of bacteria, HP microbiology and epidemiology (prevalence and geographical distribution, transmission and routes of infection).

Chapter 2. Clinical aspects of disease associated with H. Pylori describes the types of diseases (acute and chronic gastritis, peptic ulcer disease, atrophic gastritis, intestinal metaplasia, gastric cancer, gastroesophageal reflux disease) HP histopathology, diagnosis and treatment of H.P infection.

Chapter 3. Pathogenesis of HP infection analyses animal models used to elucidate the mechanisms governing disease development and the pathogenic properties of HP and the effects of treatment on the pathogenesis of H. Pylori infection. Also this chapter describes the immune response (role of antibodies in immunity, immune modulation, activation of innate immune response, resistance to phagocytosis) and the importance of host genetic polymorphism in H. pylori-related diseases.

II. OWN CONTRIBUTIONS

Chapter 4. MATERIALS AND METHODS

MATERIAL EXAMINED

The clinical study was performed on 101 patients with dyspeptic symptoms, who were sent to Bucharest University Hospital Emergency for an upper gastrointestinal endoscopy:
The first group: 52 patients (70 men, 31 women) with HP infection;
The second control group, 49 patients without HP infection but with dyseptic symptoms

HP infection was identified by serum antibody test, urea breath test, testing the presence of antigen in the seat and gastric biopsy.

STUDY METHODS

SAMPLING. Biopsic samples were obtained during the performance of gastrointestinal endoscopy. Immediately after sampling, fragments for histological examination were fixed in 10% neutral formalin solution and those for ultrastructural investigation were prefixed in 2.7% glutaraldehyde solution.

STUDY TECHNIQUES: histochemical techniques: biopsy pieces were fixed in 10% formalin and stained using haematoxylin-eosine and Van Gieson; immunohistochemical techniques; electron microscopy techniques.

Chapter 5. RESULTS

Methods of altering the gastric mucosa induced by H pylori

After examining a gastric mucosa preparation, I noticed that the severity of alternative and degenerative structural changes seems to be directly proportional with the infection level, patients with mild infection showed only isolated bacteria located in the mucus lining the surface of the mucosa; gastric epithelium appears intact without detectable changes on the examined histological preparation. Fig. 1 shows that when the number of bacteria is large enough, they act, causing visible injuries, initially superficial, but eventually deeper and deeper. Histopathological and ultrastructural investigation allowed the interception of two ways of impact and destruction of epithelial cells:
(A) attachment of H pylori at the apical pole of epithelial cells, with the widening of intercellular spaces and important issue of mucus secretion; (B) H pylori penetrates through the surface the mucosa lining cells, dissociating the intercellular junctions.

A. H pylori is attached to the apical cell in an approximately horizontal position compared to the surface of the epithelium and tightly connected to the cells. (Fig. 2)

Following these interactions, the first ultrastructural changes occur:
- thinning the border of microvilli, mucus granules become poorly electron dense
- early dilation of endoplasmic reticulum, deformation of the nuclear layer

Our observations show that, with mucus hypersecretion cells lining the mucosa surface try to protect themselves against bacterial attack. Increased mucus secretion favors bacteria which, very well adapted to the conditions of pH, mucus viscosity and its structural features, multiply even faster (Fig. 3), and appear as groups attached to the apical surface of the epithelium (Fig.4).

Note that bacteria don’t act only on the surface of mucosal epithelium, but they forward with ease in the gastric mucus from the gastric crypts where they multiply. H pylori is attached to the apical pole of the cells, which determines its swelling and mucus hypersecretion. The large amount of agglutinated mucus can be seen in the crypt lumen, encompassing many bacteria and PMN (Fig. 5).

The increased number of H pylori causes epithelial alterations – initially at the apical pole, in the end to highlighting a neutrophil inflammatory reaction, capillary hyperaemia with discrete extravasated hematite. (Fig.6)

B. Some bacteria penetrate through the surface of cells lining, "forcing" intercellular junctions.

This mode of action is suggested by the fact that some bacteria are caught among the surface epithelial cells in a particular measure that is perpendicular to the mucosal surface (Fig. 7). Bacteria first penetrate superficially, then deeper till the 1/3 level of the epithelium. At the same time, intercellular spaces widen (Fig. 8), and cells undergo progressive atrophy.
Figure 1. Bacteria (*H pylori*++) at the surface of antral gastric mucosa epithelium (HE, X20)

Figure 2. Attachment detail of *H pylori* to microvilli of apical surface (X50400)

Figure 3. *H pylori* in a large number in gastric mucus (x21000)

Figure 4. Group of bacteria that attack the apical surface of epithelium (X20000)

Figure 5. In crypt lumen, mucus that encompasses bacteria and PMN (HE, X20)

Figure 6. Abundant, inflammatory, neutrophil infiltration, capillary hyperaemia, discreet extravasated red blood cells (HE,X5)
Host body's response to H pylori infection

The primary effect of H pylori colonization is the development of active chronic gastritis well expressed in the antral level and the defining histopathological feature is the chronic inflammatory infiltrate (monocitar) and the acute inflammatory infiltrate (neutrophilic) below the surface epithelium (Fig. 9).

In the acute phase of infection there is an increased mobilization of neutrophil granulocytes, as the infection become more chronic the cellular infiltrate of the chorion becomes mixed, granulo-plasmocitar. When H pylori colonization is persistent there is a strong correlation between the distribution of acid secretion and gastritis. In subjects with normal acid secretion, H pylori colonize the gastric antral portion in particular, in which there are few acid-secreting parietal cells. This pattern of colonization is associated with the emergence of a predominantly antral gastritis. Histological evaluation of specimens
taken from the gastric body show at these subjects an inactive and limited chronic inflammatory process and a low number of bacteria which colonize only the superficial portions.

**Interaction of H pylori bacteria with gastric movement**

In addition to cellular defense reaction triggered by H pylori infection I have revealed a vascular response with different intensity: in patients with advanced infection and distructions there was a marked congestion of vessels in the shallow chorion (Fig. 10) and perivascular edema, and those of chorion deep near glands.

![Figure 9. Active chronic superficial gastritis H. pylori-positive, intestinal metaplasia (HE,X5)](image1)

![Figure 10. Ectasia with increased vascular blood stasis, perivascular edema (HE.X20)](image2)

**Highlighting histochemical and electronomicroscopic sequence:**

**chronic atrophic gastritis - intestinal metaplasia - dysplasia - gastric cancer in patients diagnosed with Helicobacter pylori.**

H pylori-induced chronic inflammation leads to the loss of normal architecture of gastric mucosa with the destruction of gastric glands and the replacement of normal mucosa with fibrous tissue and intestinal type epithelium. The risk of developing atrophic gastritis
depends on the distribution of the active chronic inflammation and how it spreads: patients with a low rate of acid secretion have a tendency to evaluate faster towards atrophy.

In chronic infection, gastric mucosal epithelium may undergo a gradual process of intestinal metaplasia: high epithelium, with an oval nucleus, situated in the third basal cell and "edge of the brush" at the apical pole. Among intestinal-type cells are distinguished caliciform cells secreting mucus, PAS - positive.

In our study we observed the process of intestinal metaplasia (Fig. 11) and ultrastructural investigation confirmed intestinal metaplasia: the edge in brush with many microvilli normally formed on their surface a well developed glicocalix with the cytoplasm containing numerous mitochondria. (Fig.12).

In our study we observed that patients, who showed a severe degree of chronic atrophic gastritis and H pylori positive, developed intestinal metaplasia and epithelial dysplasia lesions of various degrees (mild and severe) (Fig.13)

Patients with extensive intestinal metaplasia associated with intraepithelial neoplasia and the presence of sulfomucin secretory phenotype showed increased risk of progression to gastric cancer (Fig. 14).
Figure 11. Intestinal metaplasia in deeper glands (HE,X20)

Figure 12. Intestinal metaplasia: a large number of microvilli normally formed and many more mitochondria showing an intense metabolic activity (X8400)

Figure 13. Inactive chronic antral gastritis with severe epithelial dysplasia area (HE, X40)

Figure 14. Tubulo-papillary adenocarcinoma (HE, X20)
Histochemical and immunohistochemical highlighting of gastric cancer in patients diagnosed with Helicobacter pylori

I studied 10 patients with ulcerative–vegetable gastric cancer, ulcerated gastric cancer, and early gastric cancer flat type Iib and a tumor less than 2 cm, with a pediclet and antral Bössel surface.

Histologically I observed that:

- In adenocarcinoma cells 'in ring seal' is observed isolated groups of tumor cells with intracellular mucus, the nuclei are pushed to the periphery, optical clear cytoplasm with mucinous acid
- in mucosecretant adenocarcinoma is observed glands with cylindrical mucosecretant epithelium with interstitial mucin, rare gland with intestinal metaplasia (Figure 15).

Immunohistochemically I showed the following:

- CK20 with diffuse positive expression in gastric adenocarcinoma (figure 16);
- EGFR with positive expression in tubular adenocarcinoma (figure 17 and figure 18);
- PCNA with positive expression in papillary adenocarcinoma in 50% of tumor cells
- Ki67 with positive expression in approximately 20% of tumor cells nuclei
- NSE and SYN with diffuse positive expression in gastric carcinoid (figure 19 and figure 20)
- CEA zonal positive and CROMO A weak positive in gastric carcinoma
Figure 15. Mucosecretant gastric adenocarcinoma, focal intestinal metaplasia (HE, X10)

Figure 16. CK20 positive in cytoplasm of tumor cells (gastric adenocarcinoma, X40)

Figure 17. EGFR positive in pots at the front of invasion and tumoral glands (tubular adenocarcinoma, X40)

Figure 18. EGFR positive in peritumoral pots (tubular adenocarcinoma, X10)

Figure 19. NSE diffusely positive in tumor cells (gastric carcinoid, X40)

Figure 20. SYN focal positive in tumor cells (gastric carcinoid, X40)
On analyse our results presented in Chapter 5 compared with existing data in the literature.

**Chapter 7. CONCLUSIONS**

- *H. pylori* induces two ways to alter the gastric mucosa: (i) attach to the apical pole of epithelial gastric cells, (ii) dissociation of intercellular junctions and penetration among gastric cells.

- The inflammatory response triggered by infection with *H. pylori* are involved mastocyte and eosinophil cells which contribute to chronic inflammatory response.

- In addition to *H. pylori* action on the apical pole of gastric mucosa cells in, it causes also: deterioration of the chorion and a vascular response with congestion, edema and the risk of upper digestive bleeding.

- *H. pylori* induces ultrastructural changes evidenced by vacuolation of cytoplasm, swollen mitochondria, picnosis and nuclei cariolisis.

- *H. pylori* infection may cause structural and ultrastructural changes at the level of gastric mucosa, which are identified histochemical, immunohistochemical and respectively electrono-microscopic, showing with a typical sequencing the manifestation of the gastric carcinogenesis: active chronic gastritis, atrophic gastritis-metaplasia-dysplasia-adenocarcinom.
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