EXPERIMENTAL INFECTION WITH HELICOBACTER PYLORI IN RATS

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Key words: Helicobacter pylori, experimental infection, rat.

Abstract: The aim of this study was seeing if is possible to infect rats with Helicobacter pylori and to evaluate the pathogenesis of the bacteria. There was utilised an experimental group of 6 adult Whistar rats, and a control group of 4 rats. Each experimental rat was orally inoculated with positive human gastric biopsies, two times at three weeks interval. Three weeks from the second inoculation the rats were euthanised and examined (gross examination, bacterioscopy, urease rapid test and histology). In all experimental rats, not in control, urease test was positive. The exam of the gastric smears, in infected cases, showed the presence of only Helicobacter pylori–like bacteria. The histological findings consisted in chronic active superficial gastritis, apoptosis and necrosis of superficial epithelium of antrum, neutrophilic gastritis, represented by superficial epithelial alteration, ulcers, inflammatory infiltrate with mononuclear cells and granulocytes, and superficial fibrosis of lamina propria. Our results show that Helicobacter pylori is pathogen for rats, at least in experimental conditions, and that the lesions caused in rats are similar to those in humans and animal models.

INTRODUCTION

Helicobacter pylori infection represents one of the most common and medically prominent infections worldwide. Infection with this bacterium has been established as an etiologic factor in the development of peptic ulcer disease and has been associated firmly with the development of gastric neoplasia, including gastric adenocarcinomas and gastric mucosal-associated lymphoid tissue lymphomas.


Immediately following infection, H pylori causes acute gastritis characterized by neutrophils infiltration into the foveolar and surface epithelium and epithelial degenerative changes. H. pylori cause a persistent infection in the majority of infected individuals. The acute phase lasts 1 to 4 weeks and is replaced gradually by a chronic, mononuclear infiltrate in the lamina propria.

Individuals infected in early childhood are especially at risk for the development of multifocal atrophic gastritis and subsequent gastric adenocarcinoma. Elevated rates of early childhood infection in developing countries may explain, at least in part, the greatly increased prevalence of gastric adenocarcinoma in these locations. Seroepidemiologic studies indicate that infected individuals are at 6-fold increased risk for gastric MALT lymphoma (13).

There is recent evidence that Helicobacter pylori may be involved in the pathogenesis of a large number of extragastric diseases: hepatobiliary, cardiovascular, respiratory, neurological and hematological diseases. In Romania, the high prevalence of infection in
children (about 40%) compared with the prevalence in west countries (10-15%) is not entirely explained. The direct contact with pets (dogs, cats, guinea pigs) or other animals like pigs, sheep, or indirect contact with wild urban rats, could explain this high prevalence.

Dogs can be experimentally infected with \textit{H. pylori}, but there are limited sources of gastrointestinal pathology in dogs. A study reporting \textit{H. pylori-like} organism in gastric mucosa of dog suggested the possibility of the natural transmission of infection between humans and dogs (2). \textit{Helicobacter pylori} is pathogen for dogs, at least in experimental conditions and that the acute lesions caused in dogs are similar to those in others animal models – neutrophilic gastritis and ulcers (3).

\textit{H. pylori} have not been observed in pet cats. A study reporting \textit{H. pylori} in commercial vendor cats led to a suggestion that \textit{H. pylori} may be a zoonotic pathogen with transmission occurring from cats to humans (6). \textit{H. pylori} have not been isolated from the stray cats studied by El-Zataari et al (16). However, \textit{H. pylori} may be a zoonotic or anthroponotic pathogen with transmission between cats and humans. In addition, \textit{Helicobacter pylori} has been detected in tissues from piglets with gastric ulcers and from sheep milk.

The mechanism by which \textit{H. pylori} and other gastric helicobacters move from the stomach of one host to that of another remains an enigma. The fecal-oral route involving humans and pet animals is one of possibilities that must be tested. The high prevalence of gastric helicobacters in pets may have several implications. First, pets may be a source of infection for human beings since \textit{H. heilmannii} has been isolated from humans with gastric pathology (1,8) Second, the clinical significance of gastric helicobacters in cats and dogs is of interest from a veterinary viewpoint; they may cause gastric and/or related diseases. Finally, gastric helicobacters of dogs and cats may be used as an animal model for human disease.

MATERIAL AND METHODS

There was utilised an experimental group of 6 adult rats, and a control group of 4 rats. The both groups were maintained in laboratory conditions. The experimental group was infected by positive human gastric biopsies, obtained from Gastroenterology Clinic, Cluj–Napoca. Each experimental rat was orally inoculated with positive human gastric biopsies. After 3 weeks had been made a second inoculation, also with positive human gastric biopsies from the same clinic. In 3 weeks from the second inoculation the rats were euthanasied and examined macroscopically. There were collected samples for histopathology from stomach and rapid urease test. The samples were fixed in buffered formalin 10% and proceeded by paraffin technique. The used stains were Haematoxylin-Eosin, Tricrome-Masson, and polychrome blue. The microscopic exam was made by light field and phase contrast microscopy. There were realised gastric smears too, from each mucous region. The slides were stained by polychrome blue. Urease test was made in each rat by immersing of gastric mucous fragment in yellow reactive (C.P. test or “home-made” test). It was noticed the colour change of the reactive, from yellow to pink-red colour.

RESULTS

Gross examination. Three experimental rats (A,B,C) presented congestion of gastric fundic region, with small ulcers covered by coagulated blood. The mucous had a granular aspect because of blank umbilicated points.

Other experimental rat (D) presented chronic ulcers in fundic region and obvious acute ulcers and petechiae; the mucous had the same granular aspects because of prominent blank points. The rat E presented the same blank points too, which represented chronic ulcer scars.
The last experimental rat, F, have noticed small ulcers and haemorrhages with a narrow blank surrounding area in fundic gastric region; that corresponds to sub acute gastric ulcers. The control rats didn’t presented any gastric lesions or other lesions.

The rapid urease test. All tested samples collected from experimental group were positive. The colour changes in time were variable. In rats A and B, the colour changed from yellow to dark pink in almost 30 minutes. In the rat C, the yellow colour changed in bright red after 10 minutes. In rat D, the red colour appeared after 15 minutes. In rat E, appeared just a bright red point in gastric mucosa, in 15 minutes. In the last sample (rat F) the colour changed in 30 minutes.

<table>
<thead>
<tr>
<th>Sample</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
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<tbody>
<tr>
<td>Period - minutes</td>
<td>30</td>
<td>30</td>
<td>10</td>
<td>15</td>
<td>15</td>
<td>30</td>
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<tr>
<td>Colour</td>
<td>Dark pink</td>
<td>Dark pink</td>
<td>Bright red</td>
<td>Red</td>
<td>Mucosal gastric red point</td>
<td>Red</td>
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<td>Results</td>
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Gastric smear exam revealed, in all experimental rats, the presence of cocoid or curved bacteria, such us “U”, “S”, or coma form. The size of bacteria was 2-3 µm. The bacterium was founded in all gastric mucous regions, more frequent in fundic region. The bacterium morphology was the same with *Helicobacter pylori*. The colonisation degree was different from a zone to another and from rat to another one, but in all samples were noticed bacteria with *Helicobacter pylori* morphology. In all samples were noticed, exclusively, bacteria with a morphology identical with *Helicobacter pylori*. In smears from control group wasn’t noticed bacteria.

Histological exam revealed focal lesions with multiple and complex changes. The lesions were noticed in fundic and pyloric region gastric mucosa. There was encountered apoptosis in superficial epithelium, apoptosis in gland neck region, superficial epithelial necrosis, fundic and pyloric epithelial neck gland necrosis, and cells with haemosiderin because of haemorrhages. In the majority of cases, near apoptotic and necrotic cells were encountered *Helicobacter pylori*-like bacteria. Superficial epithelium was infiltrated with granulocytes and lymphocytes, associated with described epithelial lesions. Associated to chronic superficial gastritis was noticed the epithelial superficial atrophy and gastric crypts shortening, parietal vacuolisation and necrosis. In deep lamina propria, especially in pyloric area, was noticed infiltrate of lymphocytes and granulocytes, many eosinophiles; the superficially lamina propria was infiltrated with lymphocytes, granulocytes and plasmocytes. Associated to chronic gastritis was encountered cystic distension of fundic glands. The encountered lesion was chronic active superficial gastritis, especially in fundic region and rarely in antral region.

Another interesting aspect was antral and, especially, fundic ulcers. The acute ulcer morphology indicates equilibrium between necrosis and regeneration, extended till to the muscularis mucosae. In this focus, was noticed a massive destruction of fundic gastric mucosa by apoptosis, and epithelial or even lamina propria necrosis. The reminiscent lamina propria was highly inphiltrate with granulocytes, and a few lymphocytes and macrophages. There was an intense epithelial regeneration represented by young epithelial cells. Epithelial regeneration started from the gland necks produced an atypical regeneration of mucosa. In deep lamina
proper and muscularis mucosae, associated to ulcers, was encountered granulocytic infiltrate (neutrophils, eosinophiles) and fibrosis.

**DISCUSSIONS**

In this study we demonstrate that *Helicobacter pylori* experimental infection could be realised without immunosuppression. This thing could indicate the bacterial interspecific transmission possibilities and the potential germ reservoir of wild rats.

Rapports that suggest *Helicobacter pylori*-like microorganisms in gastric mucosa of dogs, suggest the possible transmission from dog to human (2). *Helicobacter pylori* are a pathogen agent in dog, at least in experimental conditions, and acute lesions induced by this pathogen agent are the same like in humans, or in animal experiments. Histology exam revealed foci of epithelial superficial necrosis in pilory antrum, superficial ulcers, and associated to epithelial necrosis was encountered neutrophilic superficial gastritis and superficial lamina propria hypertrophy. The results of the experiment indicate that *Helicobacter pylori* is pathogen in dogs, and incipient lesions are similar to those induced by the bacteria in animal experimental model, respective superficial neutrophilic gastritis and ulcers (3).

This rapport demonstrates that, lesions induced by *Helicobacter pylori* in experimental infection in rats are similar to human and canine experimental infection. Also, superficial epithelial fundic mucosa alteration, combined with chronic active inflammation that is characterised by lympho-plasmacytic proliferation, fibrosis and neutrophilic infiltrate in lamina propria, associated with the germ presence in mucosa, are similar lesions with *Helicobacter pylori* gastritis in human (3).

In developed countries, the overall prevalence of *H pylori* infection ranges from 25% to 30%. Seroprevalence increases with age, ranging from 5% to 27% in early childhood to levels exceeding 50% in adults older than 50 years. Seroprevalence studies demonstrate an acquisition rate in adults of 3% to 4% per decade (4,5,11,14,15). The prevalence of *Helicobacter* infection in adults from Romania is 60% and about 40% of children are affected. Infection occurs during childhood. The infection source (known today) is represented by infected humans (symptomatic and asymptomatic). There are some authors who have published some isolated cases of a different route of transmission: from animals to humans. In humans, close personal contact seems to enhance the transmission of *H. pylori*, which is suspected to be transmitted by oral-oral or fecal-oral routes, which are evidenced by isolation of *H. pylori* from saliva and feces.

*Helicobacter pylori* infections in animals may be an example of a reverse zoonosis. Epidemiologic studies of the relationship between *H. pylori* infection and animal contact are conflicting. It is more likely that humans are the primary reservoir of the organism. In this scenario, humans transmit the organism to animals, but might animals have the potential for transmission back to humans?

**CONCLUSIONS**

*Helicobacter pylori* can be transmitted experimentally in laboratory rats, without immunosuppression, aspect that indicate the bacterial inter-specific transmission possibility. The rats could be a reservoir and a vector for human infection with *Helicobacter pylori*. In experimental conditions, *Helicobacter pylori* are a pathogen for rats, and induce chronic active superficial gastritis, represented by superficial epithelial alteration, ulcers,
inflammatory infiltrate with mononuclear cells and granulocytes, and superficial fibrosis of lamina propria.

**BIBLIOGRAPHY**