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Overview of H. pylori infection and the mechanisms of action of amoxicillin and clarithromycin against H. pylori

Jiayu Liu

Ulink College of Shanghai, China
Jiayu.Liu01@ulink.cn

Abstract:

H. pylori infection is a highly prevalent medical condition caused by *Helicobacter pylori*. Detailed recordation of this pathogen dates back to the 20th century. According to statistics, nearly half of the world's population is infected with the bacterium so far, which indicates that the infection is a serious public health concern. *H. pylori* infection may lead to a spectrum of gastric disorders, such as chronic gastritis, gastroduodenal ulcer and gastric cancer. In addition, the infection can cause a series of extra gastric complications. As a bacterium that can survive under acidic conditions, *H. pylori* has a unique adaptation mechanism, and its virulence factors are also mentioned in this paper. Amoxicillin and clarithromycin are two frequently used antibiotics for the treatment of *H.pylori* infection. They are all produced by modifying original antibiotics and they have distinctive molecular structures. This paper introduces the mechanisms of action and targets of these two types of drugs. Common treatments for *H.pylori* infection and some special approaches for combating resistance to conventional antibiotics are also discussed in the text.

Keywords: H. pylori, Amoxicillin, clarithromycin, antibiotics.

1. Introduction

H. pylori infection is a prevalent gastrointestinal infection engendered by the bacterium *Helicobacter pylori*. The discovery and the earliest investigation of this bacterium can be traced back to the 20th century. In 1979, Robin Warren first observed the spiral-shaped bacterium in a biopsy of a patient's stomach lining and hypothesized that the infection of this bacterium might be the main cause of stomach problems. The unusual bacterium had a strong tolerance for stomach acid, which was beyond the scope of cognition of most people at that time, so many professionals thought the hypothesis was unreasonable. But in the next two years, Robin Warren and Barry J. Marshall successfully demonstrated a strong association between *Helicobacter pylori* and gastric inflammation through collaborating on experimental research, and won the Nobel Prize for Physiology or Medicine in 2005.

H. pylori infection constitutes a significant public health issue, as this bacterium resides within the alimentary canals of nearly half the global population, potentially precipitating a spectrum of gastric diseases and associated complications[1].

The way to determine *H. pylori* infection involves a carbon-13 urea breath test. During this test, the participant swallows a pill, liquid, or pudding that contains tagged carbon molecules. If the participant has an infection, carbon is released when the solution comes in contact with H. pylori in the stomach. After carbon is absorbed, it is released when the participant breathes out. A special device is used to detect the carbon molecules in exhaled air that is stored in a bag.[2]

The worldwide prevalence of *H. pylori* is approximately 44.3%. Notably, the infection rate in developing nations stands at 50.8%, significantly higher than the rate of 34.7% observed in developed countries.[3] *H. pylori* infection tends to be more common in regions with suboptimal sanitary conditions and without putting into practice separate dining systems due to the main possible transmission routes of *H. pylori* are fecal-oral transmission and oral-oral transmission.

Although most people who are infected with *H. pylori* will not have any symptoms[4], numerous gastric pathologies can be caused by the infection, including chronic inflammation, atrophic gastritis (which is a chronic inflammation that causes thinning of the stomach lining, the cells in the stomach lining mimic intestinal cells) [5], gastroduodenal ulcers and malignancies.[1] *H. pylori* infection is one of the high-risk factors for gastric cancer. According to statistical estimates, the number of gastric cancer cases worldwide reached 1.089 million in 2020, ranking fifth among all malignancies. In that same year, approximately 769,000 deaths were caused by gastric cancer, making it the fourth leading cause of cancer-related mortality across all cancer types.[6]

Extragastric complications arising from *H. pylori* infection include iron-deficiency anemia(insufficient iron absorption resulting from gastric erosion and gastrorrhagia), immune thrombocytopenic purpura(caused by abnormal response of the immune system), vitamin B12 absorption disorder(caused by deficiency of intrinsic factor synthesis due to gastric mucosa atrophy), diabetes mellitus(*H. pylori* may cause impaired function of islet beta cells by promoting cytokine production, which in turn affects blood sugar concentration), cardiovascular diseases(through long-term inflammatory stimulation, the formation of atherosclerotic plaque can be promoted and increase the risk of coronary heart disease)and certain neurological disorders(*H. pylori* may secrete cytotoxic proteins that cause nerve cell damage).[1]

To eliminate the potential risk of *H. pylori* infection, a multitude of antibiotics are available for inhibiting the growth of this microorganism. The paper describes in detail the mechanism of two commonly used antibiotics, amoxicillin and clarithromycin, against *H. pylori*.

2. Structure, adaptiveness and pathogenesis of Helicobacter pylori

Helicobacter pylori is a 0.5–1 μm wide, 2–4 μm long, short helical, S-shaped Gram-negative bacterium which is mostly found in the pyloric region of the stomach[7] and relies on flagella for movement. The diameter of a flagellum of the bacterium is about 30nm, consisting of an internal filament that is 12nm in diameter and a sheath. The flagella sheath possesses a bilayered membrane and contains fatty acids characteristic of lipopolysaccharides.[8] Compared with Gram-positive bacteria, this Gram-negative bacterium has a thinner cell wall and an outer membrane. Outer membrane proteins (OMPs), such as blood group antigen-binding adhesin (BabA) and sialic acid-binding adhesin (SabA), provide a barrier for the bacterium to resist the external environment. OMPs also have been shown to be important components for the bacterium to attach to the surface of host cells. The unique spiral structure, the presence of flagella, and outer membrane proteins accelerate the movement of *H. pylori* within the viscous gastric mucosal layers while promoting its attachment to epithelial cells.

In addition, *H. pylori* urease secretion can ease the acidic pH, creating a suitable environment for survival and replication.[9] Several genes in the genome of *H. pylori* are specialized for the production of urease, which hydrolyses urea into ammonia and carbon dioxide. The expression of urease is important for the persistence of bacteria, and urea transporters (Urel) transport urea in a pH-dependent manner to produce ammonia that buffers the bacteria's periplasm, forming a neutral layer conducive to its survival. The ammonia produced not only acts as a buffer but is also a toxic substance that causes damage to the host's cells.[9]

When *H. pylori* is able to maintain stabilization in the gastric epithelial lining, a hydrophilic, surface-exposed bacterial protein— CagA (cytotoxin-associated gene A) is injected into host cells through a type IV secretion system (T4SS), which together with VacA (a diffusible pore-forming exotoxin which is secreted by the bacterium to induce the formation of large cytoplasmic vacuoles in host cells, can effectively impair mitochondrial function and cause gastric mucosal injury), destroy gastric epithelial cells.[9] According to the presence of CagA protein, *H. pylori* can

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be divided into type I (CagA positive) and type II (CagA negative) strains. It is known that type I strains can cause more serious gastric mucosal injury and inflammation and a higher probability of gastric cancer than type II strains.[10] CagA is a crucial virulence factor of this bacterium which can interact with multiple types of proteins in cells and disrupt the normal signal transduction pathway of cells, causing a series of functional disorders of gastric epithelial cells. CagA protein is encoded by the cagA gene at the end of cag PAI (cag pathogenicity island); the protein sequence of CagA can be segmented into conserved regions and variable regions.[10] The variable region consists of several different repeat sequences. The characteristic sequence is glutamate-proline-isoleucine-tyrosine-alanine (Glu-Pro-Ile-Tyr-Ala, EPIYA) motif, which is repeated and has amino acids that are varied in number and type. When CagA is injected into cells, the tyrosine site in its EPIYA motif is phosphorylated by Src kinase and c-Abl kinase, and the phosphorylated CagA protein plays a vital role in initiating cell signaling pathway and causing cytopathia.[10]

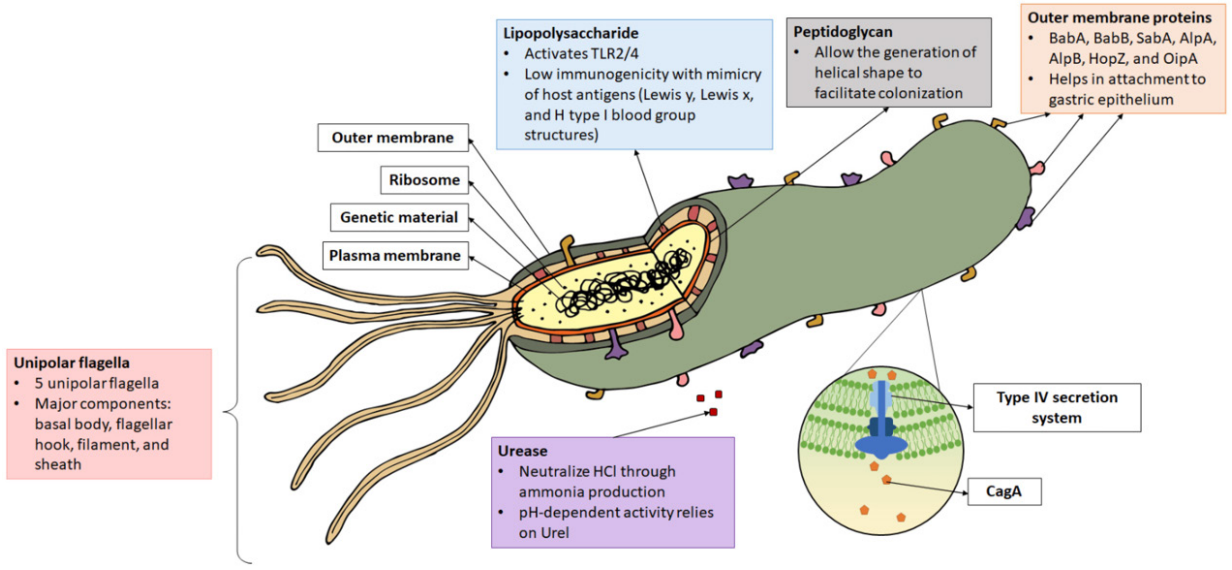

Figure 1 Structure of H. pylori.[9]

3. Introduction of amoxicillin, the mechanism of action and the target site

In 1972, Beecham Research Laboratories discovered this type of penicillin antibiotic, which belongs to beta-lactam antimicrobial drugs. It is an aminopenicillin created by adding an extra amino group to penicillin(which will be protonated in acidic media, making the compounds less acid-labile [11]) to battle antibiotic resistance, and its molecular formula is $C_{16}H_{19}N_3O_5S$.[12] The substituent at position 6 of the penam ring is a 2-amino-2-(4-hydroxyphenyl)acetamido group.

The IUPAC name of amoxicillin is (2S,5R,6R)-6-[[(2R)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid. Amoxicillin is usually taken orally, it inhibits penicillin-binding protein-1 and other high molecular weight penicillin-binding proteins. Penicillin-binding proteins are responsible for glycosyltransferase and transpeptidase reactions that lead to the cross-linking of D-alanine and D-aspartate in the bacterial cell wall. Without the action of penicillin-binding proteins, bacteria up-regulate autolysin and are unable to build and repair the cell wall, resulting in bactericidal action.[13] The basic structure of amoxicillin that plays a major role in antibacterial activity is the B-lactam ring in 6-amino-penicillanic acid, which can specifically bind to the target site(penicillin-binding proteins) on the inner membrane of *H. pylori*, inhibit the activity of bacterial cell wall mucopeptin synthetase, and thus hinder the synthesis of cell wall mucopeptin, resulting in bacterial cell wall defect and lysis of the bacterium.

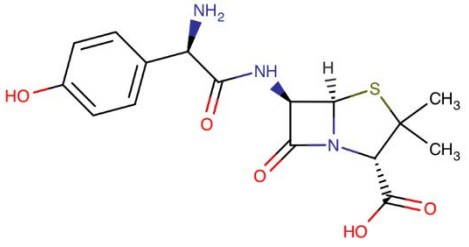

Figure 2 Structure of amoxicillin.[13]

4. Introduction of clarithromycin, the mechanism of action and the target site

Clarithromycin is the 6-O-methyl ether of erythromycin A. Its molecular structure is characterized by a 14-membered ring. It is an acid-stable macrolide antibiotic with a broad spectrum of antibacterial activity, good absorption with a wide tissue distribution and mild side effects[14], and its molecular formula is $C_{38}H_{69}NO_{13}$. The IUPAC name of clarithromycin is (3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-6-[(2*S*,3*R*,4*S*,6*R*)-4-(dimethylamino)-3-hydroxy-6-methyloxan-2-yl] oxy-14-ethyl-12,13-dihydroxy-4-[(2*R*,4*R*,5*S*,6*S*)-5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl]oxy-7-methoxy-3,5,7,9,11,13-hexamethyl-oxacyclotetradecane-2,10-dione.[15] Clarithromycin is also taken orally. It was synthesized in 1970 by the Japanese pharmaceutical company Taisho Pharmaceutical Ltd. Clarithromycin inhibits bacterial protein synthesis by binding to the bacterial 50S ribosome subunit. Binding inhibits peptidyl transferase activity and interferes with amino acid translocations during the translation and protein assembly process. Clarithromycin can be bacteriostatic or bactericidal, depending on the organism and the concentration of the drug.[15] Clarithromycin is first metabolized to 14-OH clarithromycin, which is active and works synergistically with its parent compound. Like other macrolides, it then penetrates the bacterial cell wall and reversibly binds to domain V of 23S ribosomal RNA of the 50S subunit of the bacterial ribosome, preventing translocation of aminoacyl transfer RNA and polypeptide synthesis. Clarithromycin also inhibits liver microsomal CYP3A4 isoenzymes and P-glycoproteins, an energy-dependent drug expulsion pump.[15]

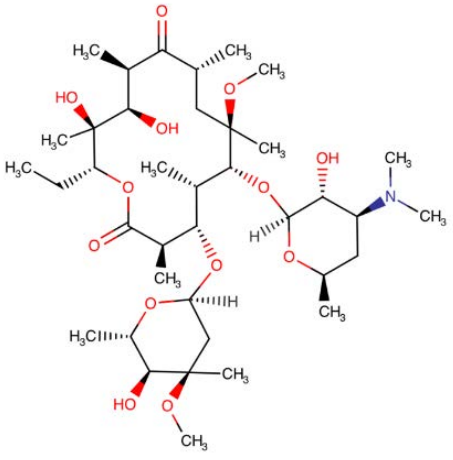

Figure 3 Structure of clarithromycin.[15]

5. Common treatments for H.pylori infection

Helicobacter pylori infections are usually treated with at least two different types of antibiotics at the same time. This helps prevent the bacterium from developing resistance to a particular antibiotic. Sequential therapy was the predominant therapeutic regimen, which is consisted of amoxicillin plus a proton pump inhibitor(can increase gastric pH by inhibiting the secretion of gastric acid and make antimicrobial therapy become more effective) for 7 days, followed by clarithromycin, tinidazole, or metronidazole plus a proton pump inhibitor for a further 7 days.[16] *Helicobacter pylori* occupies many different niches, from gastric mucus to inside the epithelial cells of the stomach. In general, a 14-day course of treatments has been proven to provide the best results, as the longer duration helps kill bacteria present in different niches.[16] The triple therapy, which consists of the combination of clarithromycin, amoxicillin, and a proton pump inhibitor, is also widely utilized.

6. Special treatments for H. pylori infection

Consider that *H. pylori* has developed resistance to some antibiotics (especially clarithromycin) and some patients are allergic to penicillin, bismuth-based quadruple therapy(consisting of a proton pump inhibitor (PPI), bismuth, metronidazole, and tetracycline, usually is required to proceed a 14-day course of treatment) can be used as an alternative one.[16] In areas where there is moderate resistance to clarithromycin but no sensitivity data are available, this may be an alternative first-line treatment. Bismuth acts synergically with several antibiotics and is not affected by clarithromycin and metronidazole resistance.[17]In patients who have failed first-line therapy, bismuth-based quadruple therapy or levofloxacin-based triple therapy are second-line options to avoid repeated clarithromycin use. Because of the persistent low resistance to amoxicillin and tetracycline, these drugs can be reused. Metronidazole has a synergistic effect with bismuth and can also be reused if the patient can tolerate it.[17] Bismuth-based quadruple therapy is deemed by many professionals as the most efficacious alternative therapeutic modality.

7. Conclusion

In summary, it's quite important to check for *H. pylori* infection in time due to long-term *H. pylori* infection may cause a variety of gastric diseases and the infection has become a widespread public health issue. Amoxicillin and

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clarithromycin are commonly used antibiotics to inhibit *H. pylori*, while the target of amoxicillin is penicillin-binding proteins on the inner membrane of the bacterium and the target of clarithromycin is domain V of 23S ribosomal RNA of the bacterial 50S ribosome subunit. Amoxicillin can hinder the synthesis of cell wall mucopeptin, resulting in bacterial cell wall defects. The mechanism of action of clarithromycin is preventing translocation of aminoacyl transfer RNA and polypeptide synthesis. *H.pylori* infection can be treated by using at least two different types of antibiotics at the same time with a proton pump inhibitor or using bismuth-based quadruple therapy and levofloxacin-based triple therapy as alternative methods.

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