Dihydroorotate Dehydrogenase as a Target for the Development of Novel Helicobacter pylori-Specific Antimicrobials

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Helicobacter pylori (H. pylori) infection is the world’s most common bacterial infection, affecting approximately 50% of the global population. H. pylori is the strongest known risk factor for stomach diseases, including cancer. Hence, treatment for H. pylori infection can help reduce the risk of these diseases. However, the emergence of drug-resistant strains of H. pylori and the occurrence of adverse effects resulting from current therapies have complicated the successful eradication of H. pylori infection. Although various antibiotics that target several bacterial enzymes have been discovered, dihydroorotate dehydrogenase (DHODH) may hold potential for the development of novel therapeutic agents with reduced toxicity and side effects. Here we review the existing literature that has focused on strategies for developing novel therapeutic agents that target the DHODH of H. pylori.

Key words Helicobacter pylori; drug resistance; dihydroorotate dehydrogenase (DHODH); pyrimidine biosynthesis

Helicobacter pylori (H. pylori) is a human gastric pathogen that causes such stomach diseases as gastritis, peptic ulcers, mucosa-associated lymphoid tissue lymphoma, and gastric cancer.1) The rate of H. pylori infection is nearly 50% worldwide, and as high as 80–90% in developing countries.2,3) Recently, clarithromycin-resistant H. pylori have been recognized by WHO as a “high-priority bacteria” for which new treatments are urgently needed (http://www.who.int/mediacentre/news/releases/2017/bacteria-antibiotics-needed/en/). Although the eradication of H. pylori using multiple antibiotics is regarded as the most effective way to prevent or cure H. pylori infection-related stomach diseases, the success rates of these eradication therapies have fallen due to the increasing prevalence of antibiotic-resistant H. pylori (Fig. 1A). According to a report by Ghotasliou et al., there is a worldwide increase in the frequency of resistance to antibiotics most commonly used to treat H. pylori infection (Fig. 1B).4) Thus, to tackle the growing problem of antibiotic resistance, new clinical regimens have been proposed to increase the efficacy of existing antibiotics.5,6) In addition, the development of new and highly selective anti-H. pylori agents is also required to avoid the emergence of new antibiotic-resistant strains and to ameliorate the side effects caused by current antibiotics, which also target the gut microflora.7)

Pyrimidines are the building blocks of DNA and RNA and are required for the growth of all living organisms. Cells can obtain pyrimidines through de novo biosynthesis and via salvage pathways. Genomic analysis of H. pylori has revealed that it possesses no orthologous pyrimidine salvage genes, which suggests that H. pylori exclusively uses the de novo biosynthesis pathway to obtain pyrimidines.8,9) Among the well-known human pathogens, H. pylori and Plasmodium falciparum (P. falciparum) depend on de novo pyrimidine biosynthesis for growth.10,11) Dihydroorotate dehydrogenase (DHODH) is a key enzyme in the de novo pyrimidine biosynthesis pathway in most prokaryotic and eukaryotic cells.12,13) It

Fig. 1. Recent Increase in the Number of Reports of Antibiotic-Resistant H. pylori and the Geographical Distribution of Resistance Rates to the Main Antibiotics

(A) A pictorial depiction of the increasing number of reports regarding the antibiotic resistance of H. pylori. PubMed was used to search the literature using “H. pylori” and “antibiotic resistance” as keywords. (B) Resistance rates to the main antibiotics used for treating H. pylori in the last five years (2009–2015). Cited from6 with the publisher’s permission.
catalyzes the fourth step in this pathway, which is the oxidation of dihydroorotate to orotate, and reduction of an acceptor (Fig. 2). Based on their cellular localization, DHODHs can be classified into families 1A, 1B, and 2. Family 1 DHODHs are cytosolic enzymes that utilize fumarate (family 1A) or NAD$^+$ (the oxidized form of nicotinamide adenine dinucleotide) (family 1B) as electron acceptors, whereas family 2 enzymes are associated with mitochondrial inner membranes in eukaryotes or plasma membranes in prokaryotes, and use respiratory quinones as electron acceptors. Because of the connection between family 2 DHODH and quinones, electrons from dihydroorotate are shuttled to the electron transport chain, which in turn produces the proton-motive force needed for ATP synthesis, thereby linking the pyrimidine de novo biosynthesis to cellular bioenergetics. DHODHs in *H. pylori*, *P. falciparum*, and humans belong to family 2.10,14,15)

Pharmacological inhibition of DHODHs has been shown to be an effective therapeutic strategy against autoimmune disorders. Leflunomide (Arava) is a Food and Drug Administration (FDA)-approved prodrug that targets human DHODH and is used to treat rheumatoid arthritis (Fig. 3A).16) Because activated lymphocytes rely on *de novo* pyrimidine biosynthesis during proliferation, the inhibition of DHODH leads to the inability of these cells to grow.17) *P. falciparum* is a protozoan parasite that causes malaria in humans and lacks the enzymes necessary for the pyrimidine salvage pathway.11) Although the WHO recommends artemisinin-based combination therapies as the first-line treatment for malaria, drug development targeting *H. pylori* DHODH remains largely unexplored. In 2002, a pyrazole compound discovered by biochemical screening was found to inhibit *H. pylori* DHODH ($K_i=26$ nM); it displayed an effective minimum inhibitory concentration (MIC) value of 3 µg/mL in *in vitro* experiments, but showed less efficacy against other Gram-negative bacteria, Gram-positive bacteria or human cells (Fig. 4).10,25) In addition, some alterations to the side chains of this compound have improved its specific inhibitory activity against *H. pylori* (Fig. 4).25) These results indicated that there are structural differences between *H. pylori*, other bacteria, and human DHODHs that are sufficient for the development of an *H. pylori*-specific DHODH inhibitor. It is worth noting that there are no reports to date describing whether these compounds are effective in eradicating *H. pylori* in humans.

*H. pylori* treatment has become more difficult than expected over the years because of the decreasing efficacy of existing...
Fig. 4. Improvement in *H. pylori* DHODH Inhibitors per Its MIC and *K*<sub>i</sub> Values

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antibiotics due to drug resistance. Although *H. pylori* is sensitive to many antibiotics in vitro, only a few can be used to eradicate *H. pylori* infection. Several proton pump inhibitors (PPIs) can control the acid levels in the stomach; however, responses to PPIs depend on patients’ genetic polymorphisms of CYP enzymes (particularly CYP2C19 and CYP3A4), which are mainly used to metabolize PPIs. Thus, it is desirable to develop compounds that are stable in acidic environments without the use of PPIs. Because the survival of *H. pylori* depends on the *de novo* pyrimidine biosynthesis pathway, DHODH is a promising target for the development of new therapeutic agents. For the development of clinically useful DHODH inhibitors for treating *H. pylori* infection, it is important to evaluate the stability of these inhibitors in acidic environments, because of *H. pylori*’s infection site in the host.<sup>28</sup> In conclusion, the development of novel drugs that target *H. pylori* DHODH is a promising strategy; however, further proof-of-concept studies are needed to fully evaluate the in vivo efficacy and toxicity of these compounds.

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**References**


