Idebenone Acts against Growth of *Helicobacter pylori* by Inhibiting Its Respiration

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Growth of *Helicobacter pylori* was inhibited by the quinones, idebenone, duroquinone, menadione, juglone, and coenzyme Q₁ at low concentrations of 0.8 to 3.2 µg/ml. Idebenone specifically inhibited *H. pylori* growth by inhibiting respiration and decreasing the cellular ATP level. The respiratory inhibition was accompanied by reduction of idebenone by the *H. pylori* cells.

Helicobacter pylori is a major cause of chronic gastritis and peptic ulcers and has been implicated in the development of gastric cancer (1, 4, 16, 19). To eradicate *H. pylori*, triple therapy consisting of amoxicillin, clarithromycin, and proton pump inhibitors has been commonly used. However, relapse and reinfection often occur, and, more seriously, treatment failure can lead to increased antibiotic resistance (11). Therefore, new drugs are needed for effective chemotherapy.

One promising group of drugs was furanonaphthoquinone analogs, which we found could inhibit *H. pylori* growth with a low MIC of around 0.1 µg/ml. However, their inhibition was not specific to *H. pylori* (12). Continuing our search led us to idebenone, duroquinone, menadione, juglone and coenzyme Q₁, which could inhibit *H. pylori* growth at MICs of 0.8 to 3.2 µg/ml, as shown in Table 1.

Among these quinone analogs, idebenone [6-(10-hydroxydecyl ubiquinone)] has no known adverse effects in humans (3, 15) and has been reported to be effective against mitochondrial diseases such as Friedreich's ataxia (10, 17). Further study of idebenone was conducted against the growth of various kinds of bacteria (Table 2). The strains of *H. pylori* and other species of bacteria and the media used to determine MIC were described in our previous report (12). Four strains of *H. pylori* showed similar MICs: 1.6 to 3.2 µg/ml. Idebenone did not inhibit the growth of other bacteria, including *Campylobacter jejuni*, with the exception of *Streptococcus pyogenes* and *Clostridium perfringens*, for which the MIC was 25 to 50 µg/ml.

The microaerophilic bacterium *H. pylori* does not catabolize saccharides (6) and does not use glucose as a preferred energy substrate (7, 8). Its potential respiratory substrates and energy sources are organic acids and amino acids. We reported previously that *H. pylori* cells could utilize amino acids such as L-serine, L-proline, and L-alanine as respiratory substrates and that these amino acids were predominantly found in human stomach juice (13).

In the present study, we examined the effect of idebenone on the respiratory activity of whole cells. Respiratory assay and

measurement of the cellular ATP level in *H. pylori* with inhibitors were carried out as described previously (14). As shown in Table 3, idebenone inhibited cellular respiration with L-proline, L-serine, and L-alanine as well as pyruvate and succinate as respiratory substrates at 50% inhibitory concentration (IC₅₀) values of 2.0 to 3.5 µg/ml, which were close to the MIC against *H. pylori* growth.

Since ATP production has been suggested to be coupled to respiration, as described previously for *H. pylori* (14), we examined the dose response of idebenone with respect to the cellular ATP level when succinate was used as a respiratory substrate. We found that idebenone dose-dependently decreased the cellular ATP level, suggesting that it can inhibit ATP production coupled to respiration (data not shown).

We next considered whether L-proline dehydrogenase, which is essential for respiratory activity, might be target of idebenone. An assay based on the 2,6-dichlorophenolindophenol-reducing activity of L-proline, described previously (13), showed that L-proline dehydrogenase was not inhibited by idebenone even at the concentration of 20 µg/ml.

Idebenone in the oxidized state has an adsorption peak at 275 nm, and the peak decreases in the reduced form (17). Assay of the reducing activity of idebenone by *H. pylori* cells showed that idebenone outside the cells was reduced time dependently when the cells were incubated with idebenone at

TABLE 1. Effect of quinone analogs on *H. pylori* ATCC 43504 growth

Quinone analog ^a	MIC ₉₀ (µg/ml) ^b
Idebenone.....	1.6~3.2
Duroquinone.....	0.8~1.6
Menadione (VK ₃).....	3.2
Juglone.....	1.6
Benzoquinone.....	25.0
Coenzyme Q ₁	3.2
Coenzyme Q ₁₀	25.0
Decylubiquinone.....	25.0

^a Coenzyme Q₁ and decylubiquinone were from Sigma Chemical Co., St. Louis, Mo.; coenzyme Q₁₀ was from MP Biochemicals, LLC, Germany; and juglone was from Tokyo-Kasei Co., Ltd. Other quinone analogs were from Wako Pure Chemicals Co., Osaka, Japan.

^b MIC₉₀, MIC which caused 90% growth inhibition, determined as described previously (12).

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TABLE 2. Effect of idebenone on growth of various kinds of bacteria

Bacteria	MIC ₉₀ (μ g/ml)
Gram positive	
Aerobes	
<i>Bacillus subtilis</i> IFO3134.....	100
Facultative anaerobes	
<i>Staphylococcus aureus</i> FAD 209P.....	>200
Anaerobes	
<i>Streptococcus salivarius</i> GIFU8326.....	>200
<i>Streptococcus pyogenes</i> E-14.....	50
<i>Streptococcus mutants</i> RIMD 3125001.....	>200
<i>Clostridium perfringens</i> NTCT4696.....	25
Gram negative	
Aerobes	
<i>Pseudomonas aeruginosa</i> IFO3445.....	>200
<i>Neisseria gonorrhoeae</i> NTCT8375.....	>200
Facultative anaerobes	
<i>Escherichia coli</i> K-12.....	>200
<i>Serratia marcescens</i> IFO12.....	>200
<i>Vibrio parahaemolyticus</i> ATCC 17802.....	>200
Microaerophiles	
<i>Campylobacter jejuni</i> NCTC11351.....	>200
<i>Helicobacter pylori</i> NCTC11637.....	1.6
SS1.....	3.2
HCM1.....	3.2
HCM3.....	3.2
Obligate anaerobes	
<i>Porphyromonas gingivalis</i> GAI7802.....	>200
<i>Wolinella succinogenes</i> DSM1740.....	>200

37°C (Fig. 1). After 2 min, almost maximum reduction of idebenone was observed and the mode of the reduction was similar to that of the respiratory inhibition (Fig. 1).

Bacteria contain two main types of quinones, ubiquinone and menaquinone, which mediate electron transfer between dehydrogenase and reductase or oxidase components of respiratory chains (18). Menaquinone is the major quinone in *H. pylori* (5, 9). Wang et al., based on work using quinones such as coenzyme Q₁, menadione, and 1,4-naphthoquinone, reported that *H. pylori* has the activity of NADPH-specific quinone reductase (20). In the present study, the cell-free homogenate obtained from sonicated *H. pylori* showed NADPH-idebenone reductase activity (data not shown). We found that idebenone, which easily crosses the cell membrane (3, 15), is reduced time dependently by *H. pylori* cells (Fig. 1). The hydrogen derived from various respiratory substrates may reduce idebenone inside the cells, which may, in turn, affect the component(s) in the respiratory chain of *H. pylori*.

Support for our hypothesis comes from the finding that the

TABLE 3. IC₅₀ of idebenone in respiration by various substrates in *H. pylori* ATCC 43504 cells

Substrate	IC ₅₀ (μ g/ml) ^a
L-Serine.....	2.0
L-Proline.....	2.8
L-Alanine.....	3.5
Pyruvate.....	2.4
Succinate.....	3.0

^a IC₅₀ values express the concentration of idebenone which shows 50% inhibition of respiration using various kinds of substrates.

FIG. 1. Inhibition of respiration and reduction of idebenone in *H. pylori* cells. (Open circles) Time course of preincubation of *H. pylori* cells with idebenone in inhibition of cellular respiration. Respiration of whole cells was monitored polarographically using a Clark-type oxygen electrode as described previously (14). Succinate was added after 0, 1, 2.5, and 5 min of preincubation of cells without or with idebenone (5 μ g/ml), and respiratory activities were determined from polarographic traces after addition of succinate, as described previously (14). The percentage of control activity was determined using the following equation: % = [(respiratory activity with idebenone) $\times$ (respiratory activity without idebenone)⁻¹] $\times$ 100. Data show means $\pm$ standard deviation obtained from triplicate experiments. (Solid circles) Time course of reduction of oxidized idebenone by *H. pylori* cells. *H. pylori* cells (2×10^8 /ml) were added to the reaction mixture containing idebenone (5 μ g/ml). After incubation of 1, 2.5, and 5 min at 37°C, cells were removed by centrifugation at $10,000 \times g$ for 10 min at 4°C, and then the A_{275} of the supernatant was measured. The percentage of control was determined using the following equation: % = [(A_{275} with cells) $\times$ (A_{275} without cells)⁻¹] $\times$ 100. Data show means $\pm$ standard deviation obtained from triplicate experiments.

cellular respiration of microaerophilic *C. jejuni*, which has a similar energy metabolic pathway to that of *H. pylori*, was not inhibited by idebenone (data not shown). *C. jejuni* cells did not reduce idebenone, and the cell-free homogenate did not show the activity of NADPH-idebenone reductase (data not shown).

Based on these observations, the action of idebenone against *H. pylori* growth is considered to be due to its inhibition of respiratory activity coupled to ATP production. Precise identification of the inhibitory target of idebenone in the respiratory chain of *H. pylori* will require further investigation using a cell-free system of *H. pylori*.

In contrast to the case for *H. pylori* observed in the present work, in human mitochondria, idebenone at 6.7 to 20 μ g/ml was found to dose dependently stimulate respiration using succinate (17). Since idebenone seems to have no serious side effects in humans (3, 10, 15, 17), our findings should be useful for developing new quinone analogs to eradicate *H. pylori*.

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