REVIEW

Korean J Intern Med 2016;31:30-35
http://dx.doi.org/10.3904/kjim.2016.31.1.30

KJIM

The antibacterial effect of fatty acids on *Helicobacter pylori* infection

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Received: October 5, 2015
Accepted: December 9, 2015

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Eradication of *Helicobacter pylori* is recommended for the management of various gastric diseases, including peptic ulcers and mucosa-associated lymphoid tissue lymphoma. Because of the increasing prevalence of antibiotic resistance, the eradication rates of antibiotic-based therapies have decreased. Therefore, alternative treatments should be considered. The antibacterial properties of fatty acids (FAs) have been investigated in various organisms, including *H. pylori*. Some FAs, particularly polyunsaturated FAs, have been shown to have bactericidal activity against *H. pylori in vitro*; however, their antibacterial effects *in vivo* remain controversial. Poor solubility and delivery of FAs may be important reasons for this discrepancy. Recently, a series of studies demonstrated the antibacterial effects of a liposomal formulation of linolenic acid against *H. pylori*, both *in vitro* and *in vivo*. Further research is needed to improve the bioavailability of FAs and apply them in clinical use.

Keywords: Helicobacter pylori; Fatty acids; Anti-bacterial agents; Liposomes; Drug delivery systems

INTRODUCTION

Helicobacter pylori is one of the most common infections reported worldwide and an important risk factor in the pathogenesis of chronic gastritis, peptic ulcers, and gastric malignancies [1]. Eradication of *H. pylori* is recommended for *H. pylori*-associated diseases, and triple therapy, consisting of a proton-pump inhibitor and two antibiotics, is the current standard regimen [2,3]. However, the rate of resistance to antibiotics has increased, and the efficacy of standard triple therapy has declined over time [4-6]. Since the failure of standard triple therapy, different regimens incorporating other antibiotics have been used, but these have demonstrated unsatisfactory eradication rates [7]. Recently, alternative treatments, such as sequential or concomitant therapy, have been applied and have exhibited promising results, but the high incidence and rapid emergence of antibiotic resistance in the treatment of *H. pylori* infection remain unresolved problems [8]. Therefore, the discovery and development of new antibacterial agents for *H. pylori* infection are needed.

Fatty acids (FAs) are carboxylic acids with long hydrocarbon chains and important components of lipids in all known organisms. FAs released from lipids can have biological effects on various cellular processes. The effect of various FAs on bacteria has been investigated in recent decades, and FAs have been reported to both inhibit growth and exhibit direct killing activity against a broad range of bacteria [9,10]. However, there has been little focus on the antibacterial activity of FAs against *H. pylori* infection. This review aims to describe the antibacterial effects of FAs by summarizing previous reports and to introduce a new formulation of FAs that improves the antibacterial activity against *H. pylori*.

pISSN 1226-3303
eISSN 2005-6648
http://www.kjim.org

EFFECTS OF FATTY ACIDS ON *H. PYLORI*

Investigations *in vitro*

FAs are characterized by the lengths of their carbon chains and their degrees of unsaturation. FAs with carbon-to-carbon double bonds are classified as unsaturated and those without double bonds as saturated. FAs can be further classified by the length of their carbon chains: short-chain FAs (< 6 carbons), medium-chain FAs (6 to 12 carbons), long-chain FAs (13 to 21 carbons), and very-long-chain FAs (> 22 carbons). The biological activities of FAs vary depending on their degree of unsaturation and length [10].

In 1989, Hazell et al. [11] reported that bovine serum albumin and catalase might reduce the toxic effects of fatty acids by adsorption to short-chain fatty acids and prevent the formation of toxic products from long-chain unsaturated FAs, thereby promoting the growth of *H. pylori*. To determine the toxic effect of long-chain polyunsaturated fatty acids (PUFAs), that group evaluated the effect of arachidonic acid (with 20 carbon atoms and 4 double bonds, or C20:4) and confirmed its bactericidal activity against *H. pylori* following 1 hour incubation in 0.1 mM arachidonic acid [12]. In an *in vitro* study by Thompson et al. [13], other unsaturated FAs also exhibited inhibitory effects on *H. pylori* growth; the inhibitory effects were greater for the FAs with a higher degree of unsaturation (oleic acid [C18:1] < linoleic acid [C18:2] < linolenic acid [C18:3] = arachidonic acid [C20:4] = eicosapentaenoic acid [C20:5]). That study also examined the intracellular distribution of ^{14}C-labeled linolenic acid and found that the majority (82%) was in the membrane of *H. pylori*, suggesting that FAs could be associated with or incorporated into the bacterial membrane and may increase its permeability. These results were confirmed by Khulusi et al. [14]. The levels of incorporation into the membrane and growth inhibition of linoleic acid were greater than those of oleic acid, indicating that the antibacterial mechanism of FAs is associated with its incorporation into the bacterial membrane. Electron microscopy revealed a distortion of the protoplasmic cylinder, as well as disruption and fragmentation of the bacterial cell membrane. Later studies showed that lauric acid (C12:0), a fully saturated FA, has bactericidal effects similar to those of PUFAs [15,16]. However, other short- or medium-chain saturated FAs exhibited little or no inhibitory activity against *H. pylori*. Interestingly, the development of spontaneous resistance to lauric acid was much lower than that to metronidazole or tetracycline. This result suggests that lauric acid also acts on the membrane of bacterial cells [15].

In a study by Sun et al. [17], the potency of FAs appeared to be related to the equivalent carbon number (ECN, or the number of carbon atoms –2 × the number of double bonds), which is used for the chromatographic analysis of lipids, in which similar ECNs imply similar retention times caused by molecules of similar size, shape, charge, or polarity. FAs with an ECN of 12, including lauric acid (C12:0), myristoleic acid (C14:1), and linolenic acid (C18:3), had the most potent antibacterial effects. This hypothesis can be applied to the previous example for arachidonic acid (C20:4) [12]. This study also reported that the bactericidal potency of unsaturated FAs increased with the degree of unsaturation, that the potency of lauric acid increased at lower pH values, and that urea and endogenous urease did not protect *H. pylori* from the bactericidal action of FAs. Docosahexaenoic acid (C22:6) decreased *H. pylori* growth in a dose-dependent manner and induced conversion from the bacillary to coccoid form, which is associated with decreased cell viability [18]. The results of the above studies on antibacterial activity are summarized in Table 1.

PUFAs have been investigated for their protective effects against various inflammatory diseases, such as inflammatory bowel disease and autoimmune diseases [19,20]. n-3 PUFAs can be converted into bioactive mediators and have anti-inflammatory properties via the counter-regulation of lipid mediators, including pro-inflammatory leukotrienes and prostaglandins [21,22]. Therefore, PUFAs may also have anti-inflammatory effects on *H. pylori*-infected gastric mucosa, in addition to their antibacterial activity. Correia et al. [18] reported that treatment with docosahexaenoic acid reduced inflammatory responses and the production of prostaglandin E2, which is associated with inflammation and tissue injury in the mouse gastric mucosa. That group also found that docosahexaenoic acid reduced bacterial adhesion to the gastric epithelium, reduced the metabolic activity of *H. pylori*, and reduced the production of interleukin-8 (IL-8), cyclooxygenase 2, and inducible nitric oxide synthase from gastric epithelial cells [23]. The inhibitory effects of PUFAs on IL-8 mRNA

Table 1. Antibacterial activities of various fatty acids

Study	Fatty acid	Incubation time	Concentration, mM	Result
Hazell et al. (1990) [12]	C20:4	1 hr	0.01	3–4 log decrease in colony forming units
Thompson et al. (1994) [13]	C18:2, C18:3	24 hr	> 0.01	Growth inhibition
			0.18	No growth
	C18:1, C18:2, C18:3, C20:4, C20:5	24 hr	0.25	Relative inhibitory potency: C18:1 < C18:2 < C18:3 = C20:4 = C20:5
Khulusi et al. (1995) [14]	C18:1, C18:2, C20:4	24 hr	0.05–5	C18:1-reversible inhibition (< 2 mM) C18:2, C20:4-irreversible inhibition (> 0.1 mM) Relative inhibitory potency: C18:1 < C18:2 < C20:4
Petschow et al. (1996) [15]	Saturated fatty acids: C4:0–C17:0	24 hr	1.0 and 5	Only C12:0 had bactericidal effect (1 mM).
Bergsson et al. (2002) [16]	C8:0, C10:0, C12:0, C14:0, C16:1, C18:1	10 min	0.15–10	6 log decrease in colony forming units: C16:1 at 0.63 mM, C12:0 at 1.25 mM, C10:0 at 2.5 mM
		1 min	0.63–5	Significant growth inhibition: C12:0 at 2.5 mM, C10:0 at 5 mM
Sun et al. (2003) [17]	C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, C16:0, C14:1, C16:1, C18:1, C18:2, C18:3	40 min	0.1–10	4 log decrease in colony forming units: C10:0 at 4 mM, C12:0 at 1 mM, C14:0 at 5 mM, C14:1 at 0.5 mM, C16:1 at 1 mM, C18:2 at > 1 mM, C18:3 at 0.5 mM
Correia et al. (2012) [18]	C22:6	6 hr	0.05–1	No growth at > 0.25 mM

and protein expression in *H. pylori*-infected cells were confirmed by Lee et al. [24]. Interestingly, the inhibitory potency of individual FAs on IL-8 expression differed. Among PUFAs, longer and more unsaturated FAs had greater anti-inflammatory effects (linoleic acid [C18:2] < arachidonic acid [C20:4] < docosahexaenoic acid [C22:6]). However, palmitic acid (C16:0), a saturated FA, did not inhibit IL-8 production.

Experiments *in vivo* and clinical trials

Although many *in vitro* studies have confirmed the antibacterial effects of FAs, the efficacy of FAs against *H. pylori* infection *in vivo* remains controversial. Based on the finding that *in vitro* PUFAs inhibited *H. pylori* growth and modified gastric mucosal injury [13,25], Duggan et al. [26] investigated the effects of orally ingested PUFAs in patients with *H. pylori*-associated duodenal ulcers. Disappointingly, dietary interventions failed to result in significant changes in either gastric colonization by *H. pylori* or prostaglandin levels after 6 weeks, despite a significant difference in linoleic and linolenic acid consumption. Contrary to this result, another study investigating the effects of dietary PUFAs in 15 patients with functional dyspepsia and *H. pylori* infection demonstrated the possibility of PUFAs as an adjuvant treatment in the eradication of *H. pylori* [27]. The 8-week supplementation of mixed oil containing PUFAs induced bacterial clearance in 53% of patients by the end of treatment and bacterial eradication in 20% of patients 6 months later. Based on an analysis of bacterial 16s rRNA from mouse feces, fish oil with high PUFA content suppressed the growth of *H. pylori* [28]. In addition, Correia et al. [18] reported that docosahexaenoic acid reduced the ability of *H. pylori* to colonize the gastric mucosa in 50% of mice, and that the combination of docosahexaenoic acid with standard triple therapy decreased the recurrence of *H. pylori* infection in an *in vivo* mouse model. This suggests that docosahexaenoic acid should not be regarded as a replacement for conventional antibiotic regimens, but it can be useful in combination therapy to reduce recur-

http://dx.doi.org/10.3904/kjim.2016.31.1.30

rence [18].

In another interesting study, the average levels of eicosapentaenoic and docosahexaenoic acids were higher in the abdominal and buttock adipose tissues of patients without *H. pylori* compared with those of *H. pylori*-positive patients, indicating that dietary FAs could inhibit the growth of *H. pylori* [29]. However, a randomized double-blind trial reported that an eradication regimen containing fish oil (pantoprazole, clarithromycin, and eicosapen) exhibited a significantly inferior *H. pylori* eradication rate compared with a conventional eradication regimen (pantoprazole, clarithromycin, and metronidazole), but it improved symptoms in patients with non-ulcer dyspepsia regardless of *H. pylori* status [30]. Recently, Khandouzi et al. [31] evaluated the effects of adding PUFAs (eicosapentaenoic and docosahexaenoic acids) to a bismuth-based quadruple therapy on both *H. pylori* eradication and inflammatory markers. PUFAs as a supportive therapy had no additive effects on *H. pylori* eradication, IL-6 levels, or total antioxidant capacity.

Fatty acids and a novel delivery system

There is a considerable discrepancy between the reported *in vitro* and *in vivo* antibacterial effects of FAs. Overall, dietary FA regimens currently are not effective enough to serve as a primary or adjuvant eradication therapy against *H. pylori* infection. However, linolenic acid, a PUFA, exerted bactericidal effects on Staphylococcus aureus *in vitro* and also reduced the numbers of this bacterium on human skin [32]. Therefore, it can be inferred that poor delivery of FAs to *H. pylori* may result in reduced or absent antibacterial effects *in vivo*. The antibacterial activity of FAs in the stomach may be reduced, because some FAs, especially long-chain PUFAs, effective against *H. pylori* have poor solubility, which is further decreased following oral administration, are sensitive to oxidation and esterification, form lipid-protein complexes, and bind to proteins or other compounds [10,33]. Previous studies have reported that esterification of oleic acid resulted in the complete loss of its inhibitory effects, and that the antibacterial activity of FAs was markedly reduced in the presence of proteins [14,34].

Liposomes are considered novel drug delivery vehicles and are widely used to deliver therapeutic agents. Because of the phospholipid bilayer structure of liposomes, liposomes can improve the solubility of FAs, protect FAs from degradation, and easily fuse with bacterial membranes, thereby delivering their entrapped FAs into the bacterium [35-37]. Obonyo et al. [38] developed a liposomal nanoformulation of linolenic acid (LipoLLA) and evaluated its bactericidal activity against *H. pylori in vitro*. LipoLLA had a bactericidal effect on both the spiral and coccoid forms of the bacterium and eradicated various clinical strains, regardless of their antibiotic-resistance status. This group also investigated the antibacterial mechanism of LipoLLA against *H. pylori* [39]. LipoLLA rapidly killed *H. pylori* within 5 minutes, and the antibacterial activity of LipoLLA was considerably more potent than that of liposomal oleic acid (C18:1), which was consistent with previous studies using FAs. They also found that LipoLLA increased the permeability of both the outer and inner plasma membranes of *H. pylori* by measuring the uptake of 1-N-phenylnaphthylamine and detecting the release of adenosine triphosphate from bacterial cells, respectively. Structural changes to the bacterial membrane upon LipoLLA treatment were observed by both transmission and scanning electron microscopies.

Recently, the anti-*H. pylori* efficacy of LipoLLA was evaluated in a mouse model [40]. After the oral administration of LipoLLA, linolenic acid accumulated within the gastric mucus layer and a significant portion was retained for up to 24 hours. To evaluate its *in vivo* therapeutic efficacy, *H. pylori*-infected mice were treated with linolenic acid, LipoLLA, or standard triple therapy (omeprazole, clarithromycin, and amoxicillin). While linolenic acid failed to exhibit antibacterial activity, LipoLLA had *in vivo* efficacy superior to that of the standard triple therapy and resulted in reduced levels of *H. pylori*-induced proinflammatory cytokines, including IL-1β, IL-6, and TNF-α. In a toxicity test, LipoLLA had no effect on body weight, gastric histopathology, or gastric mucosal integrity in mice, indicating that LipoLLA has excellent biocompatibility.

CONCLUSIONS

As described above, *in vitro* studies have revealed that *H. pylori* is susceptible to FAs, but clinical and *in vivo* studies have reported diverse biological effects of ingested FAs. However, because some FAs have powerful

bactericidal effects, with a different mechanism of action from those of most conventional antibiotics, and inhibitory effects on gastric inflammation, they have great potential as novel antibacterial agents against *H. pylori*. Recently, a liposomal formulation was applied to improve the stability and delivery of FAs, which revealed promising anti-*H. pylori* effects, both *in vitro* and *in vivo*. Therefore, further studies are necessary to improve the bioavailability of FAs, and additional clinical trials using upgraded formulations of FAs are needed.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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