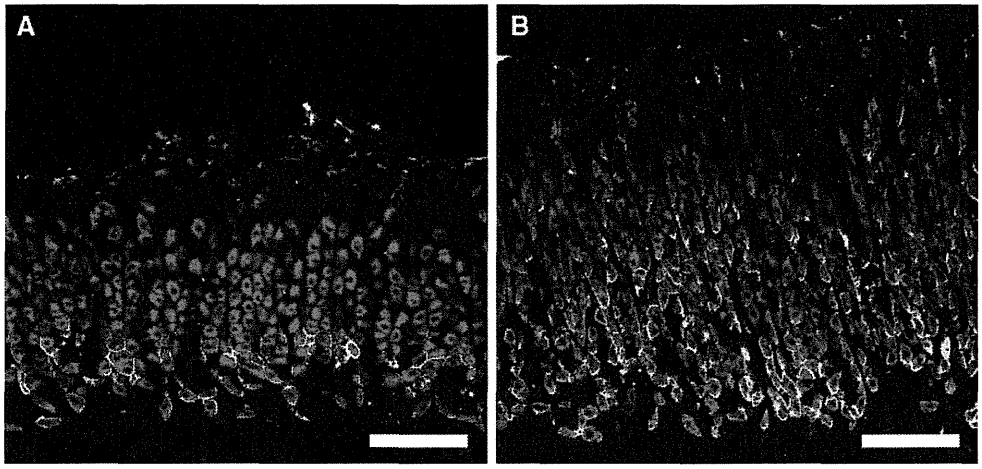

Fig. 3 Immunofluorescent double staining for AQP4 and H^+/K^+-ATPase. **a** Representative immunofluorescence for AQP4 and H^+/K^+-ATPase in the control mouse; AQP4 is expressed on the basolateral membrane of the parietal cells in the lower part of the fundic glands (AQP4-positive parietal cells). **b** Representative immunofluorescence for AQP4 and H^+/K^+-ATPase in the LPZ-treated mouse; the extension of AQP4-positive parietal cells towards the neck region of the fundic glands is clearly observed. H^+/K^+-ATPase-positive parietal cells become smaller in size (×200, *bars* = 100 μm)

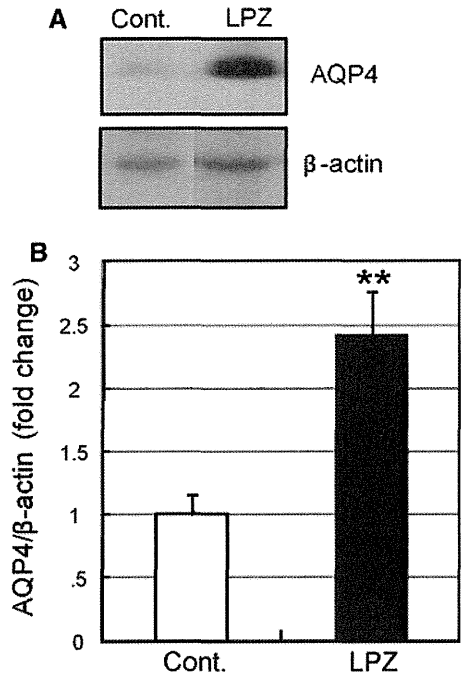

Fig. 4 Increased expression of AQP4 protein after PPI administration. **a** The protein expression level of AQP4 was analyzed by Western blotting in the LPZ-treated mice and the control mice. β-actin was used as the loading control. **b** Band quantitation for the results of Western blotting; significantly increased expression of the AQP4 protein was observed in the LPZ-treated mice (means ± SE, ** $p < 0.01$, $n = 10$ LPZ-treated mice, $n = 11$ control mice)

Delayed Mucous Neck-to-Zymogenic Cell Differentiation After PPI Administration

In normal fundic gland, the mucous neck cells secrete Muc6 and pepsinogen. As mucous neck cells migrate towards the bases of fundic glands, they transdifferentiate into zymogenic cells, which do not secrete Muc6 but continue to secrete pepsinogen in abundance [35, 36]. In the present study, while Muc6-positive cells were strictly localized to the neck region of the fundic glands in the control mice (Fig. 6a), Muc6-positive cells were detected even in the basal region of the fundic glands in the LPZ-treated mice (Fig. 6c). On the other hand, while the pepsinogen-positive cells were mainly localized to the lower region of the fundic glands in the control mice (Fig. 6b), pepsinogen-positive cells were observed even in the neck region of the fundic glands in the LPZ-treated mice (Fig. 6d). The number of cells co-expressing Muc6 and pepsinogen seemed to be increased at the base of the fundic glands. The results of quantitative RT-PCR revealed that Muc6 mRNA expression was significantly increased (Fig. 6e), while pepsinogen mRNA expression was significantly decreased (Fig. 6f) in the LPZ-treated mice. These results suggest that zymogenic cell differentiation was disrupted, and mucous neck cells, co-expressing Muc6 and low amount of pepsinogen, were increased after PPI administration.

Discussion

In the present study, the PPI-treated mice exhibited striking changes in the components of the epithelial lining of the gastric fundic glands. Although a number of previous studies have reported that PPI treatment leads to mucosal hyperplasia with hypergastrinemia [37–39], mucosal cystic dilatation has not been mentioned yet. However, similar hyperplastic change is also observed in H_2R-null mice [40],

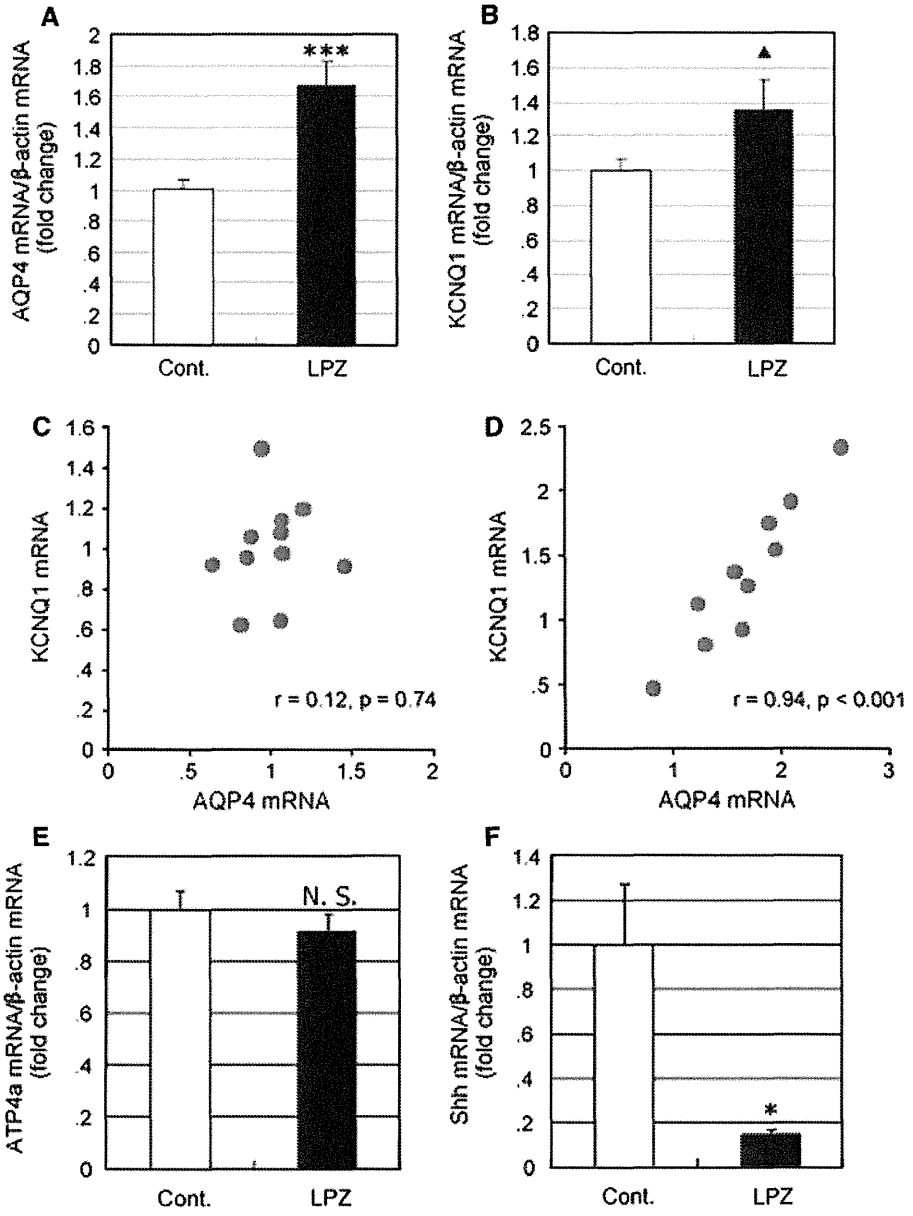

Fig. 5 Enhanced expression of AQP4 and KCNQ1 mRNA after PPI administration. **a** Significant increase in the expression of AQP4 mRNA is observed in the LPZ-treated mice. **b** A tendency towards increase in the expression of KCNQ1 mRNA is observed in the LPZ-treated mice. **c** No correlation between the expressions of AQP4 mRNA and KCNQ1 mRNA is observed in the control mice. **d** Significant correlation between the expressions of AQP4 mRNA and KCNQ1 mRNA is observed in the LPZ-treated mice. **e** No difference in the expression of H^+/K^+-ATPase α subunit mRNA is observed between in the LPZ-treated mice and control mice. **f** Significant decrease in the expression of Shh mRNA is observed in the LPZ-treated mice (means ± SE, *** $p < 0.001$, * $p < 0.05$, ▲ $p < 0.1$, $n = 10$ LPZ-treated mice, $n = 11$ control mice)

transgenic mice overexpressing transforming growth factor-α (TGF-α) [41], which stimulates gastrin secretion [42], and mice with parietal cell-specific deletion of Shh. All of these mice have hypergastrinemia and hypochlorhydria. In addition, Fukushima et al. [39] reported that fundic glands in LPZ-treated mice were very similar to those in H_2R-null mice. Zavros et al. [43] reported that achlorhydria reduced gastric elimination of bacteria and resulted in bacterial overgrowth in gastrin knockout mice and omeprazole-treated mice, suggesting that bacterial overgrowth is one of the possible causes of the mucosal hyperplasia. Hypergastrinemia and hypochlorhydria would be an essential condition for the development of hyperplastic and cystic alterations in fundic glands.

In the control mice, AQP4-positive parietal cells were localized in the basal part in the fundic glands, while KCNQ1-positive parietal cells were distributed at a slightly lower level than the H^+/K^+-ATPase-positive parietal cells in the control mice. These results suggest functional and developmental diversity between lower parietal cells and upper parietal cells as has been observed in other reports [44–46]. In the LPZ-treated mice, the AQP4-positive parietal cells increased in number and extended toward the upper region of the glands, suggesting that the number of lower parietal cells was increased after PPI administration.

One of the functions of the lower parietal cells is to provide water and potassium to the upper part of the fundic glands. Potassium is recycled by H^+/K^+-ATPase for proton

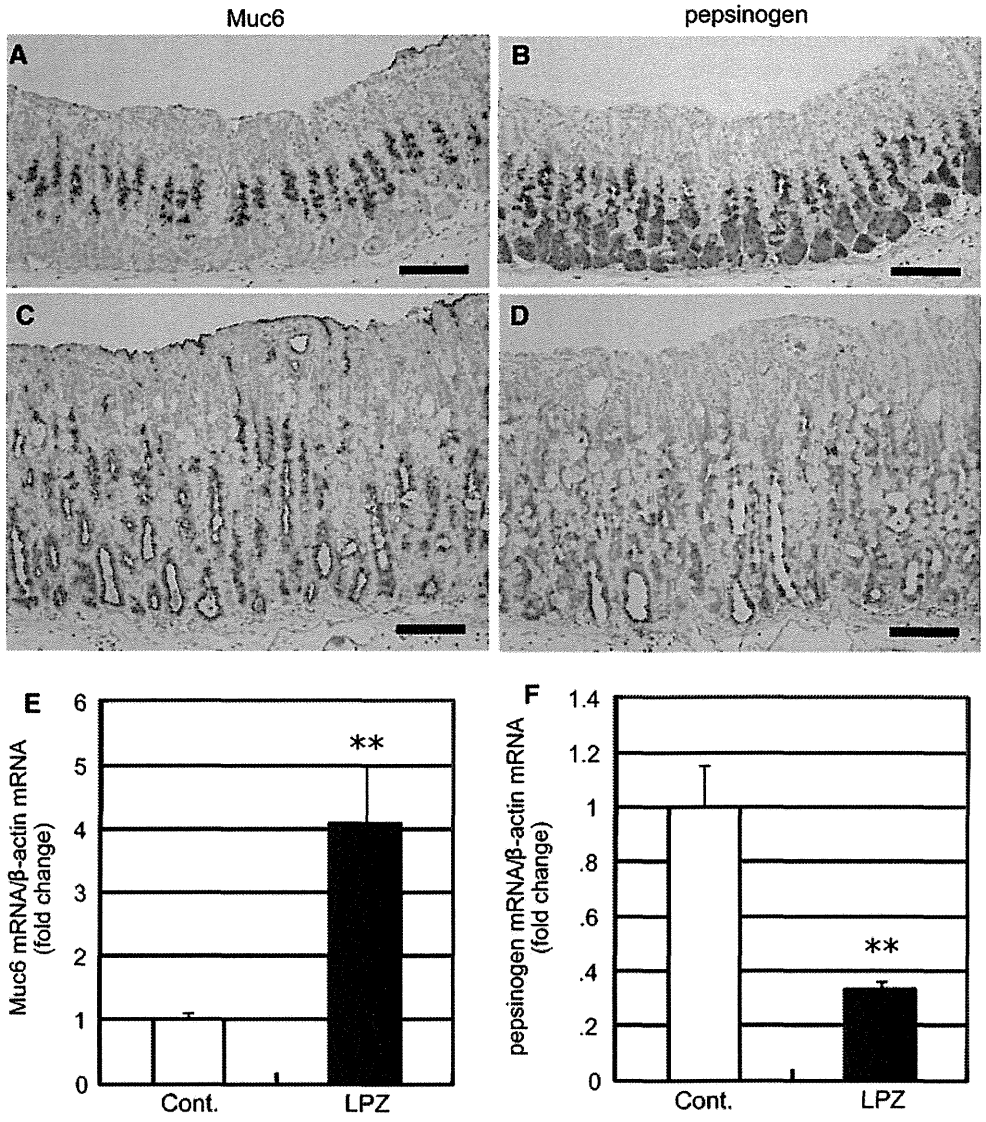

Fig. 6 Impaired mucous neck-to-zymogenic cell lineage differentiation after PPI administration. **a** Representative immunohistochemistry for mucin-6 (Muc6) in the control mouse; Muc6-positive cells are exclusively localized in the neck region of the fundic glands. **b** Representative immunohistochemistry for Muc6 in the LPZ-treated mouse; Muc6-positive cells are observed extending to the basal region of the fundic glands. **c** Representative immunohistochemistry for pepsinogen in the control mouse; pepsinogen-positive cells are mainly localized in the basal region of the fundic glands. **d** Representative immunohistochemistry for pepsinogen in the LPZ-treated mouse; pepsinogen-positive cells are observed extending to the neck region of the fundic glands (×200, *bars* = 100 μm). **e** Significant increase in the expression of Muc6 mRNA is observed in the LPZ-treated mice. **f** Significant decrease in the expression of pepsinogen mRNA is observed in the LPZ-treated mice (means ± SE, ** $p < 0.01$, $n = 10$ LPZ-treated mice, $n = 11$ control mice)

secretion. PPIs are known to suppress not only acid secretion but also the volume of gastric juice [47]. Therefore, the elevation of AQP4 and KCNQ1 expression in the gastric fundic glands observed after PPI administration is considered to occur as compensation for the loss of gastric juice volume and gastric acid secretion.

The significantly positive correlation between the expressions of AQP4 and KCNQ1 mRNA suggests that both may be enhanced by the same intracellular signal after PPI administration. Gastrin stimulation is known to increase the abundance of *c-fos* and *c-jun* mRNA expression and also of the AP-1 (fos/jun)-dependent transcriptional activity in the parietal cells [48]. In addition, the sequences of the AP-1-binding domain are contained within the first 1,000-bp promoter region of both AQP4 and KCNQ1. Therefore, it is possible that gastrin stimulation enhances the mRNA expressions of AQP4 and KCNQ1 by activating AP-1 after PPI administration.

Shh mRNA expression was significantly suppressed in the stomach of the LPZ-treated mice, suggesting that suppressed Shh expression causes hyperproliferation of the lower parietal cells, as well as delayed mucous neck-to-zymogenic cell differentiation. Recent studies have shown that delayed mucous neck-to-zymogenic cell differentiation was observed in mice with parietal cell-specific deletion of Shh [32]. We also reported that suppressed Shh expression caused abnormal mucous neck-to-zymogenic cell lineage differentiation in the *H. pylori*-colonized stomach of Mongolian gerbils [27, 28] and in H_2R-null mice [29]. Zavros et al. [49] reported that gastrin stimulates the processing of Shh peptide by increasing acid production by the parietal cells. The increase in acid facilitates the intramolecular conversion of pepsinogen to the active protease pepsin. Pepsin subsequently cleaves Shh to the active form [49]. Therefore, a consequence of hypochlorhydria is the absence of activated pepsin available to

process Shh to the active form in the parietal cells [49]. Reduction in the availability of the active Shh leads impaired parietal cell differentiation and mucous neck-to-zymogenic cell differentiation, as well as decreased Shh and pepsinogen expression.

Although it remains unclear whether the parietal cell differentiation and mucous neck-to-zymogenic cell differentiation are impaired in human sporadic FGPs, cystic hyperplasia and without inflammatory cell infiltrations, observed in the LPZ-treated mice, is consistent with the histological character of FGPs. Previous reports suggested that PPI-induced FGPs arise as a result of impaired glandular flow secondary to mechanical obstruction of the glands caused by parietal cell hyperplasia [50]. However, the results of the present study suggest that the altered localization and expression of proteins associated with water or potassium transport in the fundic glands may also contribute to the excess fluid secretion. Further histological investigations are needed to clarify the pathogenetic mechanisms underlying the development of FGPs.

In conclusion, a significant increase in the number of AQP4 and KCNQ1-positive parietal cells in the fundic glands, with a hyperplasia and formation of multiple cystic dilated glands, and delayed mucous neck-to-zymogenic cell lineage differentiation were observed in murine gastric mucosa after PPI administration. These findings suggest that unbalanced water flow, induced by both AQP4 and KCNQ1 overexpressions, and impaired parietal cell and mucous neck-to-zymogenic cell differentiation may be responsible for the development of FGPs in the stomach of long-term PPI users.

Acknowledgments The authors thank Dr. Yasushi Fukushima for providing us with the rabbit anti-mouse pepsinogen C antibody (mPep) for this study. Preliminary data from the present study were presented at the Research Forum, Digestive Disease Week 2009 held in May 2009 in Chicago. This study was supported in part by the grant of the strategic basis on research grounds for non-governmental schools from Ministry of Education, Culture, Sports, Science and Technology (S0901008, to H.S.). This study was also supported in part by the Keio Gijuku Academic Development Funds (to H.S.).

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Original Article J. Clin. Biochem. Nutr., 46, 259–264, May 2010

H. pylori-Eradication Therapy Increases RUNX3 Expression in the Glandular Epithelial Cells in Enlarged-Fold Gastritis

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Received 28 December, 2009; Accepted 15 January, 2010; Published online 10 April, 2010

Summary ***Helicobacter pylori* (HP)-eradication therapy increases Runt domain transcription factor 3 (RUNX3) expression in the glandular epithelial cells in enlarged-fold gastritis. The aim of this study is to evaluate expression of the RUNX3 protein, the product of a gastric tumor suppression gene, and mutagenic oxidative stress in human gastric mucosal specimens obtained from patients with HP-induced enlarged-fold gastritis. Methods. RUNX3 expression was immunohistochemically scored and the degree of the mucosal oxidative stress was directly measured by the chemiluminescense (ChL) assay in the biopsy specimens. Results. RUNX3 expression was detected in the gastric epithelial cells. HP-eradication significantly increased RUNX3 expression in the glandular epithelium of the corpus, however, no change was observed in those of the antrum. A fourfold higher mucosal ChL value was observed in the corpus as compared with that in the antrum. HP-eradication significantly decreased the mucosal ChL values in both portions of the stomach to nearly undetectable levels. Conclusion. The glandular epithelium is exposed to a high level of carcinogenic oxidative stress and shows low levels of expression of the tumor suppressive molecule, RUNX3; however, this expression was restored after HP-eradication, suggesting the high risk of carcinogenesis associated with HP-induced enlarged-fold gastritis of the corpus.**

***Key Words*: *H. pylori*, RUNX3, gastritis, oxidative stress**

Introduction

Gastric cancer is the most prevalent gastrointestinal (GI) malignancy in Japan, while in other developed countries, the prevalence of colon cancer is higher than that of gastric cancer. Recent clinical studies have clearly demonstrated that patients with chronic gastritis associated with *Helicobacter pylori* (HP) infection have an approximately three-fold higher risk of developing gastric cancer than those without HP infection [*1*]. Furthermore, eradication of HP infection by antibiotics has been shown to be associated with a definite decrease in the risk of gastric cancer [*2*, *3*]. Thus, while the carcinogenic role of HP is now widely accepted, the underlying pathogenetic mechanism remains to be clarified. Recently, enlarged-fold gastritis has attracted much attention, because the risk of carcinogenesis associated with this type of gastritis has been suggested to be much higher than that associated with atrophic gastritis, the commonly observed type of chronic gastritis in the Japanese population [*4*]. This type of gastritis is characterized by enhanced inflammation of the corpus mucosa, however the

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mechanism involved in the carcinogenic potential of this type of gastritis has not been fully elucidated.

Oxidative stress, now acknowledged as one of the major carcinogenic process, directly modifies genetic nucleotides, followed by activation of many tumor suppressor genes [*5*]. On the other hand, the Runt domain transcription factors (RUNX) have also been linked to gastric cancer development [*6*]. Among the members of the RUNX family, RUNX3 has been proposed as a gastric tumor suppressor gene and its activity has been shown to be influenced by DNA methylation [*7*].

This study was aimed at clarifying the carcinogenic mechanism of enlarged-fold gastritis by measuring the levels of oxidative stress and RUNX3 expression in the gastric mucosa of patients with HP-induced enlarged-fold gastritis.

Methods

Patients and sampling

Thirteen patients with HP-induced enlarged-fold gastritis (fold width ≥5 mm) who visited the Gastroenterology Division of the National Hospital Organization Tokyo Medical Center with upper GI symptoms were enrolled in this study. All of the subjects provided written informed consent for this study, and the study protocol was approved by the Ethics Committee of Tokyo Medical Center. Upper GI endoscopy was performed in the patients. Two biopsy specimens were obtained from the antrum and two from greater curvature of the upper corpus. One of these was processed for histological assessment (Hematoxylin-Eosin stain (HE) and immunohistochemistry for RUNX3) and the other was used for assay of oxygen-free radical production. Eradication for HP was performed using routine one week course of triple therapy with proton pump inhibitor, clarithromycin, and amoxicillin. Success of eradication therapy was confirmed by ^{13}C-urea breath test.

Histological assessment

Immunohistochemistry for RUNX3 was performed according to a previously described method. [*8*] The histological assessment was performed in a blinded manner, and the degree of staining in the surface epithelial cells and glandular epithelial cells was scored as follows: intensity of gastric RUNX3 expression was graded in the epithelial cells of the mucosa as follows: 0 (negative), 1 (<33% cells showing positive staining), 2 (33–66% cells showing positive staining), or 3 (>66% cells showing positive staining). The degree of inflammatory cell infiltration was scored on the HE-stained sections according to the Updated Sydney System [*9*].

Oxygen-derived free radical measurement

Luminol-dependent chemiluminescence (ChL) assay was used for direct assessment of oxygen-derived free radical production from the gastric mucosa, according to a previously reported method [*10*, *11*]. Briefly, biopsy specimens (approximately 10 mg in wet weight) were placed, immediately after sampling, in a scintillation vial containing 0.5 ml of Eagle's minimum essential medium (MEM; pH 7.4) and 20 μg of luminol. The average ChL value (count/10 s/mg wet weight) for the first 5 min was used for the analysis.

Statistical analysis

All the data were expressed as mean ± SD and analyzed by Wilcoxon's rank sum test. Significance was set at $p<0.05$.

Results

Endoscopic appearance

The characteristic endoscopic features of enlarged-fold gastritis are shown in Fig. 1. Before the HP eradication, enlarged folds with thick mucus were observed in the greater curvature at the corpus, which clearly improved after HP eradication therapy.

RUNX3 expression in the gastric mucosa

The results of immunohistochemistry for RUNX3 protein in the gastric mucosa are illustrated in Fig. 2 (antrum) and Fig. 3 (corpus). In the antrum, RUNX3 protein expression was observed in the epithelial cells as well as the infiltrating leukocytes. Although the nuclei of epithelial cells were counterstained with hematoxylin, strong RUNX3 expression was still observed in some nucleus. While high immunoreactivity was observed in the infiltrating leukocytes, dramatic weakening of the staining intensity was observed after HP eradication. High level of the RUNX3 expression was also observed in the glandular epithelium in the corpus, which further increased following HP eradication.

Figs. 4 and 5 show the immunohistochemical scores for RUNX3 expression in the epithelial cells. Semi-quantitative analysis demonstrated that the RUNX3 expression in the surface epithelium was unchanged following HP eradication (antrum: 1.15 ± 0.55 to 1.00 ± 0.41, n.s., corpus: 1.31 ± 0.63 to 1.08 ± 0.28, n.s.). While no increase of the protein expression in the glandular epithelium was also observed in the antrum (1.38 ± 0.65 to 1.54 ± 0.52, n.s.), that in the corpus significantly increased following HP eradication (1.85 ± 0.55 to 2.54 ± 0.52, $p<0.01$).

Table 1 shows the leukocyte infiltration score as assessed according to the Updated Sydney System. Significant decrease in the number of infiltrating leukocytes (polymorphonuclear and mononuclear cells) was observed following HP eradication.

Fig. 6 illustrates the ChL activity of the gastric mucosa,

Fig. 1. Endoscopic images before (A) and after (B) HP eradication in patients with enlarged-fold gastritis. HP eradication was followed by a reduction of the enlarged folds with thick mucus.

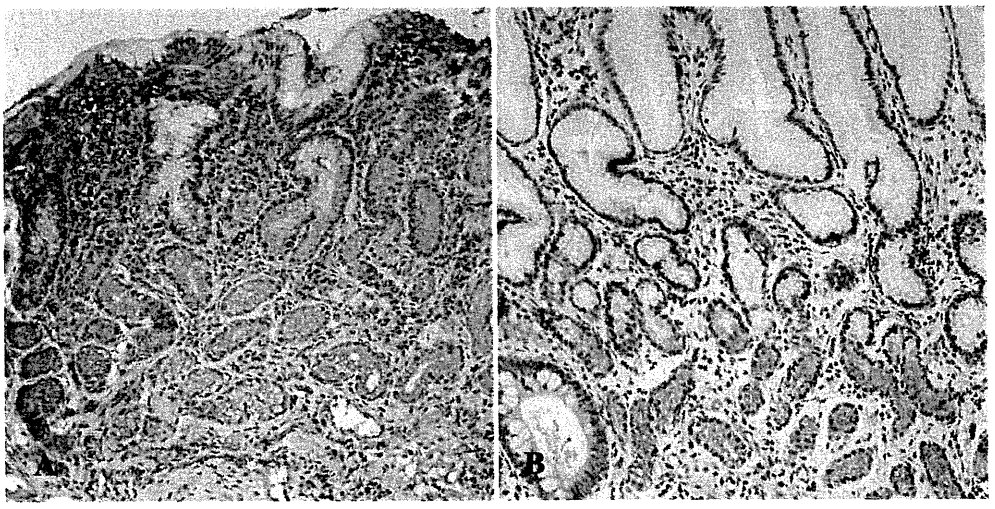

Fig. 2. Immunohistochemistry for RUNX3 protein expression in the antral mucosa pre-(A) and post-(B) HP eradication (counterstaining was also done with hematoxylin). Pre-eradication, RUNX3 expression was observed in epithelial cells as well as infiltrating leukocytes, and the strong expression was observed in the nuclei of the epithelial cells. Post-eradication, no significant change was observed in the surface and glandular epithelium.

which is a direct measure of oxidative stress. The ChL value was fourfold higher in the corpus mucosa as compared with that in the antral mucosa. Significant decrease of the ChL values to undetectable levels was observed in both portions of the gastric mucosa after HP eradication (antrum: 72.7 ± 110 to 2.89 ± 0.94, $p<0.01$; corpus: 309 ± 251 to 3.90 ± 1.92, $p<0.01$).

Discussion

Gastric carcinoma is reported as the first or second leading cause of cancer-related death in Japan. HP infection now generally accepted as one of the major factors involved in the development of gastric carcinoma. This bacterium reportedly modifies the cellular functions related to cell growth, which is mainly regulated by the cagA-protein [*12*].

Nishibayashi *et al.* reported detecting enlarged-fold gastritis (fold width ≥5 mm) in 81% of patients with gastric carcinoma, while it was detected in only 46% of HP-positive controls. Furthermore, mucosal levels of 8-OHdG, another measure of oxidative stress, were higher in specimens from enlarged-fold gastritis than those in the controls [*13*]. The finding in the present study of enhanced levels of oxidative stress as measured by tissue-associated ChL activity lend

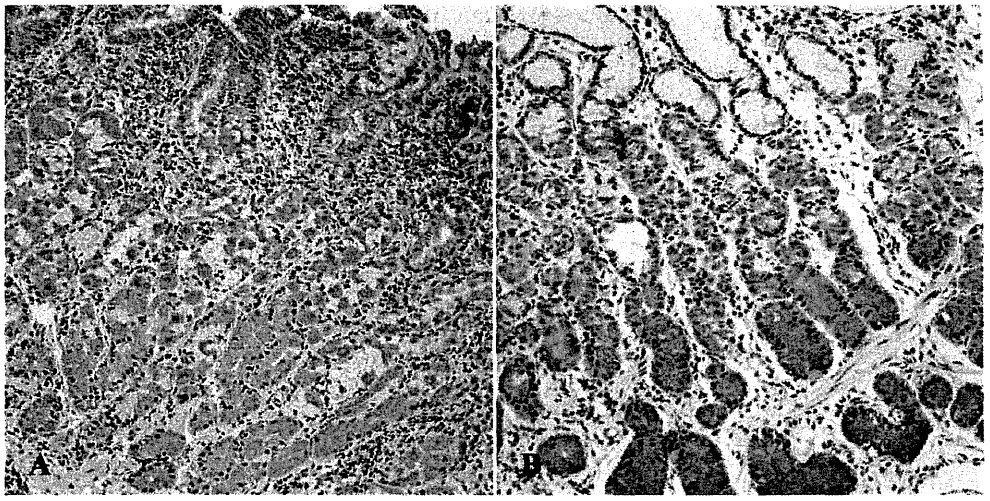

Fig. 3. Immunohistochemistry for RUNX3 protein expression in the corpus mucosa pre-(A) and post-(B) HP eradication (counterstaining was also done with hematoxylin). Pre-eradication, strong RUNX3 expression was observed in the epithelial cell as well as infiltrating leukocytes. Post-eradication, RUNX3 expression was not changed in the surface epithelial cells, while the increase was observed in the glandular epithelium.

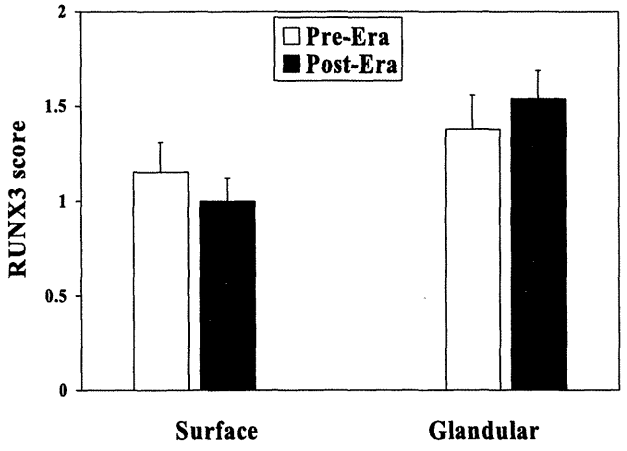

Fig. 4. Histological score for RUNX3 expression in the antral epithelial cells pre- and post-HP eradication. The protein expression in the surface and glandular epithelial cells was unchanged after HP eradication.

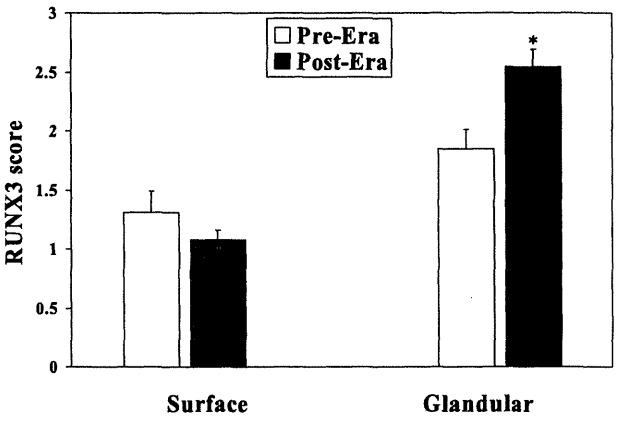

Fig. 5. Histological score for RUNX3 expression in the corpus epithelial cells pre- and post-HP eradication. RUNX3 expression in the glandular epithelial cells increased significantly after HP eradication (*$p<0.01$), while that in the surface epithelium was unchanged.

Table 1. Alterations of the histological score pre- and post-HP eradication in patients with enlarged-fold corpus gastritis.

	Antrum		Corpus	
	PMN	Mo	PMN	Mo
Pre-eradication	1.23 ± 0.73	1.92 ± 0.76	2.00 ± 0.82	2.23 ± 0.60
Post-eradication	0.46 ± 0.66	1.31 ± 0.95	0.46 ± 0.88	0.62 ± 0.87
p value	n.s.	n.s.	$p<0.01$	$p<0.01$

($n = 13$) PMN: polymorphonuclear cell, Mo: mononuclear cell.

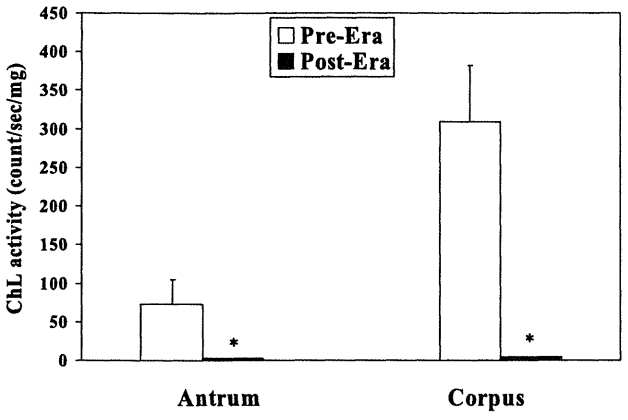

Fig. 6. Chemiluminescence (ChL) activity of the gastric mucosa (antrum and corpus) pre- and post-HP eradication. Marked ChL activity was observed in the corpus mucosa, which decreased dramatically after HP eradication. (*$p<0.01$ compared to each pre-eradication value)

support to these previous reports. Our previous data demonstrated that the corpus ChL activity in HP-positive non-enlarged-fold gastritis (DU and GU) was approximately 70% lower than that in enlarged-fold gastritis [*10*]. Since this enhanced ChL activity was dramatically reduced by eradication of HP, it was considered that elevated levels of oxidative free radical production may be one of the possible carcinogenic mechanisms resulting in gastric cancer. Significant reduction in the degree of inflammatory cell infiltration (polymorphonuclear and mononuclear cells) was also observed (antrum: 30–60%, corpus: 70–75% reduction) following HP eradication, although the magnitude of this decrease was much lower than that of ChL, suggesting that enhanced oxidative stress caused by HP infection may be regulated by not only the number of inflammatory cells, but also by other factors, including the activity of each leukocyte and tissue anti-oxidant molecules.

The Runt domain transcription factors (RUNX1, 2 and 3) have been reported to play key roles in developmental pathways in mammalian cells. Among this gene family, RUNX3 has been proposed as a gastric tumor suppressor gene. Enhanced expression of RUNX3 has been demonstrated to inhibit gastric carcinoma cell growth *in vitro*, as well as tumorigenicity and metastasis [*14*, *15*]. Friedrich *et al.* [*16*] examined RUNX3 protein expression in HP-infected non-cancerous gastric mucosa and found that the protein expression was prominently detected in the infiltrating leukocytes in the lamina propria, but not in the human gastric epithelium, as confirmed by a low mRNA expression in quantitative reverse transcriptase-polymerase chain reaction (RT-PCR). However, Nakase *et al.* [*17*] demonstrated RUNX3 expression in the normal epithelial cells of the remnant stomach by in situ hybridization as well as RT-PCR. Ito *et al.* [*8*] performed immunohistochemical staining of RUNX3 in the normal human stomach epithelial cells with R3-6E9, and found that while the chief cells and surface epithelial cells were strongly stained, the parietal cells showed only weak staining. In the present study, RUNX3 expression was clearly detected in the gastric epithelium as well as infiltrating leukocytes. Furthermore, semi-quantitative analysis revealed no significant change of RUNX3 expression in the surface epithelial cells, whereas a significant increase of the protein expression was observed in the glandular epithelium of the corpus, which is composed of chief cells and parietal cells, after HP eradication.

RUNX3 expression has often been reported to be downregulated in human gastric carcinoma owing to epigenetic silencing by promoter hypemethylation [*7*]. Kim *et al.* [*18*] demonstrated RUNX3 methylation in 8.1% of chronic gastritis specimens, 28.1% of intestinal metaplasia specimens, 27.3% of gastric adenoma specimens and 64% of gastric carcinoma specimens, suggesting increase of RUNX3 methylation with progression of the lesion along the path of multistep gastric carcinogenesis. Kitajima *et al.* [*19*] reported that RUNX3 methylation is induced by HP infection, and that subsequent loss of RUNX3 expression may affect gastric carcinogenesis. Miyazaki *et al.* [*20*] found a decrease of the immunoreactivity for E-cadherin in HP-positive patients with enlarged-fold gastritis as compared with that in HP-negative patients, and suggested that this reduction might be due to the high methylation percentage of E-cadherin.

DNA methylation is closely associated with free radical injury [*21*], and methylation of E-cadhelin has been shown to be enhanced by reactive oxygen species (ROS) [*19*]. In the present study, extensive increase of the oxidative stress in the corpus mucosa was demonstrated, thus RUNX3 expression may be partially downregulated by oxidant-induced DNA methylation. Further study including direct assessment of methylation process is necessary to clarify this issue.

In the present study, RUNX3 immunoreactivity was restored in the corpus glandular epithelium (probably chief cells) after HP eradication, while those in the other epithelium were unchanged. It is postulated that greater RUNX3 gene methylation may be induced in the glandular epithelium than in the surface epithelium by HP infection, resulting in a relatively lower expression level of RUNX3 in the glandular epithelium than in the surface epithelium, lending support to the hypothesis that carcinogenic transformation may occur more frequently in the glandular epithelium than in the surface epithelium in HP-induced enlarged-fold gastritis.

In conclusion, the low level of RUNX3 expression in the glandular epithelium and high level of oxidative stress in the corpus mucosa may contribute to the high risk of carcinogenesis associated with enlarged-fold gastritis.

Acknowledgment

R3-6E9, a monoclonal antibody directed against RUNX3, was kindly provided by Prof. Yoshiaki Ito, Institute of Molecular and Cell Biology, Singapore. This work was partially supported by a Grant-in-Aid for Exploratory Research from the Japan Society for the Promotion of Science (JSPS) (No. 19659057, to H.S.).

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Helicobacter pylori eradication therapy

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Helicobacter pylori infection is the main cause of gastritis, gastroduodenal ulcers and gastric cancer. *H. pylori* eradication has been shown to have a prophylactic effect against gastric cancer. According to several international guidelines, the first-line therapy for treating *H. pylori* infection consists of a proton pump inhibitor (PPI) or ranitidine bismuth citrate, with any two antibiotics among amoxicillin, clarithromycin and metronidazole, given for 7–14 days. However, even with these recommended regimens, *H. pylori* eradication failure is still seen in more than 20% of patients. The failure rate for first-line therapy may be higher in actual clinical practice, owing to the indiscriminate use of antibiotics. The recommended second-line therapy is a quadruple regimen composed of tetracycline, metronidazole, a bismuth salt and a PPI. The combination of PPI–amoxicillin–levofloxacin is a good option as second-line therapy. In the case of failure of second-line therapy, the patients should be evaluated using a case-by-case approach. European guidelines recommend culture before the selection of a third-line treatment based on the microbial antibiotic sensitivity. *H. pylori* isolates after two eradication failures are often resistant to both metronidazole and clarithromycin. The alternative candidates for third-line therapy are quinolones, tetracycline, rifabutin and furazolidone; high-dose PPI/amoxicillin therapy might also be promising.

Helicobacter pylori infection is one of the most prevalent infectious diseases worldwide, affecting an estimated 40–50% of the world population. *H. pylori* has been identified as a group 1 carcinogen by the WHO and is associated with the development of gastric cancer. In experimental studies, *H. pylori* eradication has been demonstrated to have a prophylactic effect against gastric cancer [1–4]. In human beings, the beneficial effect of *H. pylori* eradication in reducing gastric cancer incidence has been reported [5–7]. The indications for *H. pylori* eradication as proposed by an international consensus of experts (Maastricht III Consensus Report) are listed in Box 1 [8,9]. The revised Maastricht guidelines endorsed all the previous indications for *H. pylori* treatment.

First-line therapy for *H. pylori* infection is generally accepted to consist of a proton pump inhibitor (PPI; standard dose twice daily [b.i.d.]) or ranitidine bismuth citrate, plus two antibiotics, namely clarithromycin (500 mg b.i.d.) and amoxicillin (1 g b.i.d.), administered for 7–14 days. Metronidazole (500 mg b.i.d.) has also been used as an alternative to amoxicillin [8,10]. However, according to a number of recent meta-analyses, even with the recommended regimens, *H. pylori* eradication failure is still seen in approximately 20% of patients. This issue is becoming a cause for concern owing to the indiscriminate use of antibiotics.

Zullo *et al.* assessed the eradication rate of a new sequential treatment regimen compared with conventional triple therapy for the eradication of *H. pylori* infection. A total of 1049 dyspeptic patients were studied prospectively. *H. pylori*-infected patients were randomized to receive 10-day sequential therapy (rabeprazole [20 mg b.i.d.] plus amoxicillin [1 g b.i.d.] for the first 5 days, followed by rabeprazole [20 mg b.i.d.], clarithromycin [500 mg b.i.d.] and tinidazole [500 mg b.i.d.] for the remaining 5 days) or standard 7-day therapy (rabeprazole [20 mg b.i.d.], clarithromycin [500 mg b.i.d.] and amoxicillin [1 g b.i.d.]). Higher eradication rates were found with the sequential regimen compared with the standard regimen (intention to treat [ITT]: 92 vs 74%, $p < 0.0001$; per protocol: 95 vs 77%, $p < 0.0001$). Higher eradication rates were also evident in patients with peptic ulcer disease and nonulcer dyspepsia. In both treatments, compliance was similar (>90%), as was the rate of side effects, which were mild [11]. A pooled-data analysis of all studies on the sequential regimen was performed by Zullo *et al.* [12]. Overall, more than 1800 patients have been treated with the sequential regimen. Such a therapy was superior to 7–10-day triple therapies in pediatric, adult and elderly patients, achieving an eradication rate constantly higher than 90% at ITT analysis. The sequential regimen is a novel, promising therapeutic approach.

Review

Future Microbiology

Keywords

eradication ▪ *Helicobacter pylori* ▪ third-line therapy

future medicine part of fsg

Box 1. Indications for *Helicobacter pylori* eradication.

- Duodenal ulcer
- Gastric ulcer
- Atrophic gastritis
- Gastric mucosa-associated lymphoid tissue lymphoma
- Nonulcer dyspepsia (functional dyspepsia)
- Uninvestigated dyspepsia (in areas with a prevalence of >10%)
- Following resection of gastric cancer
- First-degree relatives of patients with gastric cancer
- Unexplained iron-deficiency anemia
- Idiopathic thrombocytopenic purpura
- Before the commencement nonsteroidal anti-inflammatory drug therapy
- Patients receiving long-term aspirin therapy
- At the patient's request (after a discussion of the risk and benefits)

Bacterial resistance

Helicobacter pylori resistance is an important factor involved in the failure of treatment. The prevalence of primary resistance of *H. pylori* to clarithromycin has been reported to range from 2.2 to 24% in different countries [13–18]. It is reported to be lower in northern (approximately 3%) compared with southern Europe (>16.9%) (Figure 1) [19–27].

The prevalence of *H. pylori* resistance to metronidazole has been reported to range from 8 to 80% in different countries. The prevalence is much higher in developing countries (>60%) than in developed countries (Figure 2) [26,28].

However, the impact of resistance to clarithromycin and metronidazole on the success of *H. pylori* eradication treatment is very different. While a good correlation between bacterial resistance to clarithromycin and eradication failure has been shown to exist, this is not the case for metronidazole [29]. Therefore, we recently developed a useful predictor of the response to metronidazole-containing regimens by combining the minimal inhibitory concentrations of both amoxicillin and metronidazole and the results of the urea breath test before the treatment [30].

The prevalence of primary resistance of *H. pylori* to quinolones has been reported to range from 2 to 22% in different countries [14,19,31–36]. The prevalence of quinolone resistance is reported to be relatively higher in Japan, Korea and Italy (15–22%), and to be very low in China and Egypt (approximately 2%). We reported a high resistance rate (47.9%) to gatifloxacin (8-methoxy fluoroquinolone) of *H. pylori* strains isolated after eradication failure in Japan [37]. Resistance to quinolones is easily acquired, and the resistance rate is relatively high in countries with a high rate of usage of these drugs.

The prevalence of the resistance to amoxicillin, tetracycline and rifampicins fortunately remains low. In most studies, it is less than 2%, with the exception of Bangladesh (6.6%) for amoxicillin and Bulgaria (5.2%) for tetracycline.

These findings suggest that regional-specific treatment regimens based on local antibiotic resistance may improve eradication rates. The third Maastricht guidelines recently recommended local reference centers to measure antibiotic resistance rates within countries to improve eradication [8].

Second-line eradication therapy

Second-line therapy has been extensively reviewed by several authors. Most authors concur that culture is not required before the start of second-line therapy after failure of first-line therapy [38]. Assessment of *H. pylori* sensitivity to antibiotics may be useful only after failure of second-line therapy. As second-line therapy, Maastricht II–2000 Consensus Report recommends a quadruple therapy regimen composed of bismuth (120 mg four-times daily [q.i.d.]), tetracycline (500 mg q.i.d.), metronidazole (500 mg three-times daily [t.i.d.]) and an antisecretory agent (standard-dose PPI b.i.d.), administered for a minimum of 7 days. Further trials have shown that substitution of the PPI and bismuth compound in the quadruple therapy by ranitidine bismuth citrate also yields good results, with an eradication rate ranging between 5 and 57%. The failure of second-line quadruple therapy is associated with its discontinuation because of the high incidence of adverse effects (6–68%). However, bismuth is no longer available in several countries, which limits the possibility of the quadruple therapy.

Recently, Gisbert *et al.* conducted a meta-analysis of studies comparing levofloxacin-based rescue regimens with quadruple therapy for *H. pylori* eradication failures. The meta-analysis showed better results with levofloxacin than with the quadruple combination (81 vs 70%; odds ratio [OR]: 1.80; 95% CI: 0.94–3.46). Meta-analysis showed fewer adverse effects with levofloxacin than with quadruple regimen, both overall (19 vs 44%; OR: 0.27; 95% CI: 0.16–0.46) and regarding severe adverse effects (0.8 vs 8.4%; OR: 0.20; 95% CI: 0.06–0.67). A 10-day combination of levofloxacin–amoxicillin–PPI constitutes an encouraging second-line alternative [39].

For patients with eradication failure to a first-line clarithromycin-based regimen, PPI-based triple therapy with amoxicillin and

metronidazole is a good alternative option in places where bismuth compounds are not available [40], such as in Japan.

Third-line eradication therapy

Currently, a standard third-line therapy still remains to be established, and European guidelines recommend culture before the selection of a third-line treatment based on the microbial antibiotic sensitivity. *H. pylori* strains isolated after two eradication failures are often resistant to both metronidazole and clarithromycin. Therefore, inclusion of these two drugs is not recommended in third-line regimens. The alternative candidates for third-line therapy are quinolones, tetracycline, rifabutin and furazolidone; high-dose PPI/amoxicillin therapy has also shown promise (Table 1) [41,42].

Quinolone-based triple therapy

Quinolones exert their antimicrobial activity by inhibiting the enzyme DNA gyrase. This enzyme, in addition to relaxing supercoiled DNA, introduces negative supercoils into the DNA, causing the bacterial chromosome to be maintained in a negatively supercoiled state. In addition, the enzyme is involved in DNA replication, recombination and transcription. Several studies have demonstrated that the 'quinolone resistance-determining region' of the *gyrA* gene plays a critical role in the resistance of *H. pylori* to quinolones [37]. We recently designed a rapid test based on an allele-specific PCR to detect *gyrA* mutations [43].

Gisbert *et al.*, in a prospective multicenter study, administered a third-line levofloxacin-based regimen to 100 patients with a history of two consecutive *H. pylori* eradication failures. Patients with failure of a first trial of omeprazole–clarithromycin–amoxicillin and a second trial of omeprazole, bismuth, tetracycline and metronidazole (or ranitidine bismuth citrate with these antibiotics) were enrolled. A 10-day regimen consisting of omeprazole (20 mg b.i.d.), levofloxacin (500 mg b.i.d.) and amoxicillin (1 g b.i.d.) was administered. The eradication rates as determined by per-protocol (PP) and ITT analyses were 66% (95% CI: 56–75) and 60% (95% CI: 50–70), respectively. Adverse effects occurred in 25% of the patients, and consisted mainly of metallic

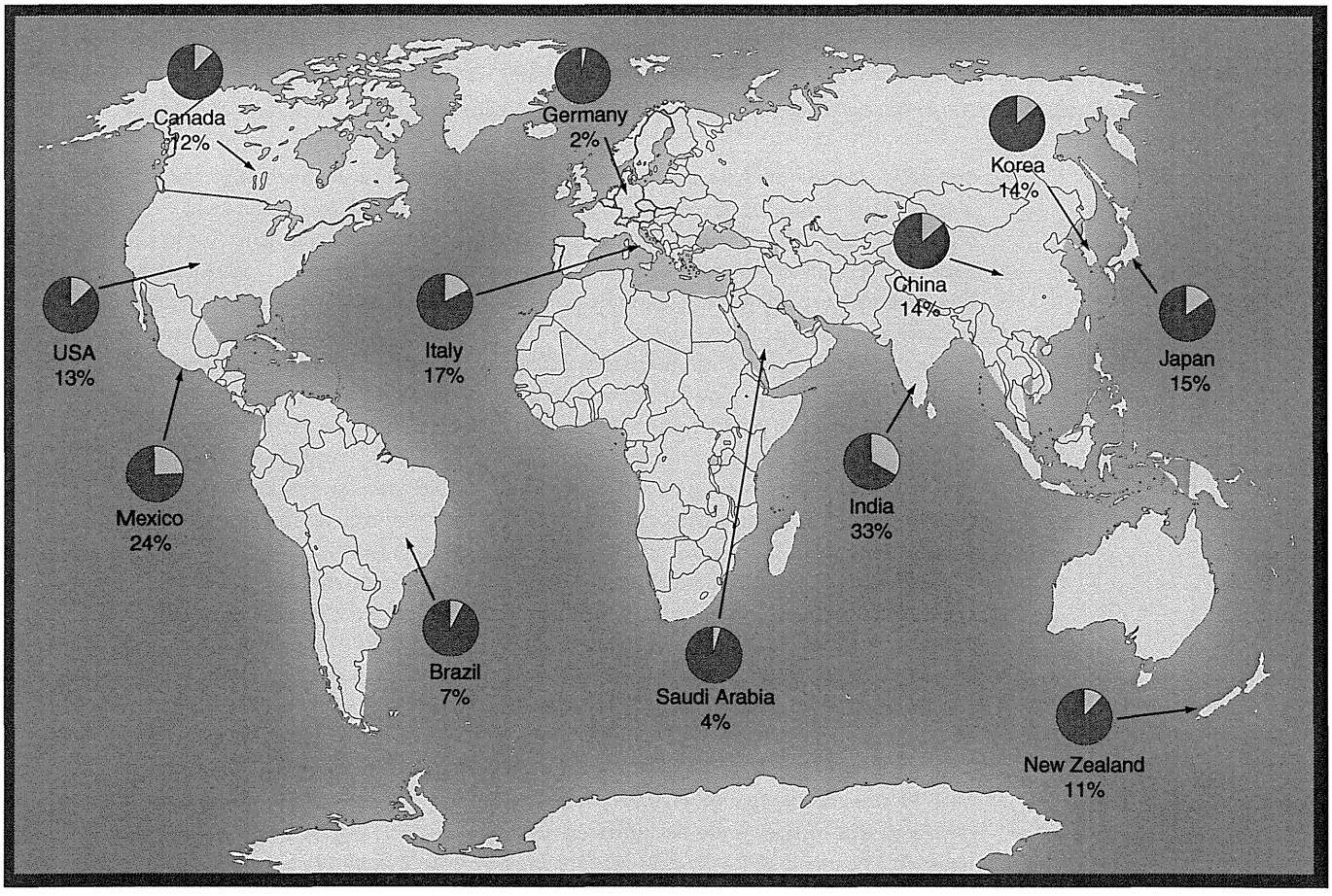

Figure 1. Primary resistance of *Helicobacter pylori* to clarithromycin in different countries.

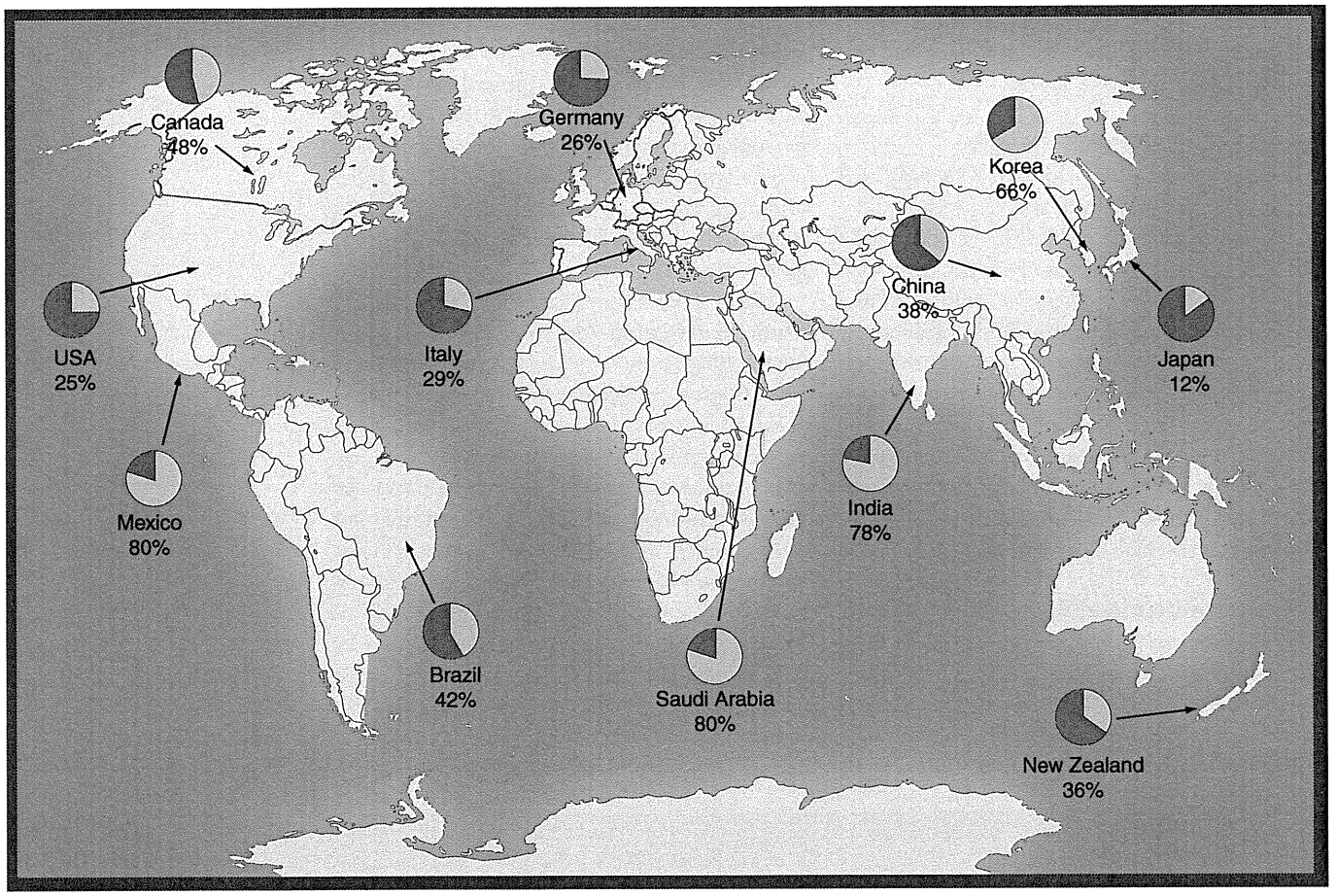

Figure 2. Primary resistance of *Helicobacter pylori* to metronidazole in different countries.

taste (8%), nausea (8%), myalgia/arthralgia (5%) and diarrhea (4%). No severe side effects were reported. Levofloxacin-based rescue therapy may represent an encouraging empirical third-line strategy for patients with multiple previous *H. pylori* eradication failures [44].

Furthermore, Gisbert *et al.* compared rifabutin and levofloxacin rescue regimens in patients with two consecutive *H. pylori* eradication failures. Patients with two failed eradication attempts received 10 days' treatment with either rifabutin (150 mg b.i.d.) or levofloxacin (500 mg b.i.d.), plus amoxicillin (1 g b.i.d.) and omeprazole (20 mg b.i.d.). In total, 20 patients received rifabutin and 20 received levofloxacin. The cure rate as determined by PP analysis was 45% (95% CI: 26–66) in the rifabutin group and 81% (95% CI: 57–93) in the levofloxacin group ($p < 0.05$). The eradication rates as determined by ITT analysis were 45% (95% CI: 26–66) and 85% (95% CI: 64–95), respectively ($p < 0.01$). This study demonstrated that a triple levofloxacin-based rescue regimen administered for 10 days is more effective than a rifabutin-based triple regimen after two previous *H. pylori* eradication failures [45].

Gatta *et al.* evaluated the eradication rate of a 10-day levofloxacin-based triple therapy as third-line treatment. A total of 151 patients with persistent *H. pylori* infection after two treatments were studied. Susceptibility testing was also performed. Patients received a standard dose of PPI twice daily, levofloxacin (250 mg b.i.d.) and amoxicillin (1 g b.i.d.), for 10 days. Approximately 76% (95% CI: 68.8–82.3) and 85% (95% CI: 77.5–89.7) of patients were eradicated according to ITT and PP analysis, respectively. Eradication rates of the strains showed as 92% (95% CI: 83.2–96.7) of those resistant to both metronidazole and clarithromycin but susceptible to levofloxacin [46].

Hsu *et al.* designed a prospective study to assess the efficacy of levofloxacin, amoxicillin, bismuth and rabeprazole quadruple therapy as third-line treatment for *H. pylori* infection. The patients were 37 consecutive *H. pylori*-infected patients with a history of failure of standard first- and second-line treatments who received a 10-day quadruple therapy comprising rabeprazole (20 mg b.i.d.), bismuth subcitrate (300 mg q.i.d.), amoxicillin (500 mg q.i.d.) and levofloxacin

(500 mg once daily [q.d.]). Successful *H. pylori* eradication was achieved in 84% of patients, as determined by both ITT and PP analyses. The findings of this study suggested that a 10-day regimen of levofloxacin and amoxicillin-based quadruple therapy is well tolerated and yields a high eradication rate as a third-line empirical treatment regimen for *H. pylori* infection [47].

Recently, novel quinolones have been developed, and superior *in vitro* activity of gatifloxacin over that of levofloxacin against *H. pylori* has been reported. Furthermore, garenoxacin (des-fluoro[6] quinolone) and sitafloxacin show fourfold or greater activities than gatifloxacin [48]. We recently investigated the efficacy of gatifloxacin-based triple therapy as a third-line eradication treatment for *H. pylori*, administered after assessment of the susceptibility of the organisms to gatifloxacin and the presence of *gyrA* mutations [49]. A 7-day regimen of gatifloxacin (400 mg q.d.), amoxicillin (500 mg q.i.d.) and rabeprazole (10 mg q.i.d.) was administered to 11 patients. Successful eradication of *H. pylori* was achieved in 63.6% of the patients, as assessed by both ITT and PP analyses. The eradication rate was 100% in the patients infected with gatifloxacin-susceptible bacteria and/or bacteria without *gyrA* mutations, but only 33.3% in those infected with gatifloxacin-resistant bacteria or bacteria with *gyrA* mutations. This difference in the eradication rate between patients infected with gatifloxacin-susceptible and -resistant bacteria was statistically significant ($p < 0.05$). Our data suggested that the selection of gatifloxacin for third-line therapy should be based on the results of drug susceptibility testing or *gyrA* mutational analysis. Unfortunately, gatifloxacin has been withdrawn from the market due to increased risk of dysglycemia associated with its administration.

Rifabutin-based therapy

Rifabutin is a spiropiperidyl derivative of rifamycin-S, an antituberculous compound. Rifabutin inhibits the β-subunit of *H. pylori* DNA-dependent RNA polymerase encoded by the *rpoB* gene [50].

Carro *et al.* evaluated the efficacy of a rifabutin-based triple therapy [51]. A total of 92 consecutive patients diagnosed as having *H. pylori* infection resistant to two previous treatment regimens were treated with pantoprazole, rifabutin and amoxicillin for 10 days. The eradication rate was 62.2% as determined by PP analysis and 60.8% as determined by ITT analysis. Only two patients were excluded because of the appearance of adverse events related to the treatment.

Table 1. Third-line *Helicobacter pylori* eradication therapy.

Patients (n)	Regimen	Duration of therapy days	Eradication rate (%)		Ref.
			ITT	*PP*	
100	Omeprazole 20 mg b.i.d. Levofloxacin 500 mg b.i.d. Amoxicillin 1 g b.i.d.	10	60	66	[44]
37	Rabeprazole 20 mg b.i.d. Levofloxacin 500 mg q.d. Bismuth 300 mg q.i.d. Amoxicillin 500 mg q.i.d.	10	84	84	[47]
67	PPI b.i.d. Rifabutin 150 mg b.i.d. Amoxicillin 1 g b.i.d.	10	72	76	[52]
20	Omeprazole 20 mg b.i.d. Rifabutin 150 mg b.i.d. Amoxicillin 1 g b.i.d.	10	45	45	[45]
10	PPI b.i.d. Furazolidone 200 mg b.i.d. Amoxicillin 1 g b.i.d.	7	60	60	[53]
42	Omeprazole 40 mg q.i.d. Amoxicillin 750 mg q.i.d.	14	75.6	83.8	[56]
89 (culture guided)	Omeprazole 20 mg b.i.d. Bismuth 120 mg b.i.d. Doxycycline 100 mg b.i.d. Amoxicillin 1 g b.i.d.	7	91	92	[59]

b.i.d.: Twice daily; ITT: Intention to treat; PP: Per protocol; PPI: Proton pump inhibitor; q.d.: Once daily; q.i.d.: Four-times daily.

Van der Poorten *et al.* designed a prospective study to assess the efficacy of a rifabutin-based triple therapy [52]. Patients referred after first or subsequent treatment failure were prescribed rifabutin-based triple therapy consisting of a PPI at the standard dose, amoxicillin (1 g b.i.d.) and rifabutin (150 mg b.i.d.), for 10 days. Among the 67 patients, the eradication rate of *H. pylori* was 76% (48/63) as determined by PP analysis and 72% (48/67) as determined by ITT analysis. When used as second-line therapy, an eradication rate of 95% (18/19) was achieved as compared with that of 68% (30/44) when it was used after two or more previous treatment failures ($p = 0.03$). Adverse events were reported in 10% of patients. The findings of this study demonstrate that rifabutin-based triple therapy is well tolerated and that the eradication rate is acceptable for a third-line therapy.

Nevertheless, rifabutin is expensive, not available in many countries and is associated with a high incidence of side effects (e.g., leukopenia, thrombocytopenia and myelotoxicity). It has been suggested rifabutin be reserved for the treatment of multiresistant *Mycobacterium tuberclosis* strains.

Furazolidone-based therapy

Furazolidone is a broad-spectrum nitrofuran, that exerts activity against Gram-negative and Gram-positive bacteria and protozoa by inhibiting their enzymes. Strains resistant to furazolidone are rare and the likelihood of the appearance of resistance to this drug is as low as that to bismuth compounds or amoxicillin.

Qasim *et al.* evaluated the efficacy and safety of rifabutin- and furazolidone-based therapy [53]. Rifabutin-based triple therapy yielded an eradication rate of 38% in 34 patients receiving third-line therapy. On the other hand, a triple regimen comprising furazolidone (200 mg b.i.d.), amoxicillin (1 g b.i.d.) and standard dose of PPI (standard-dose b.i.d.) administered for 7 days yielded an eradication rate of 60% in 10 patients with failure of first-line, second-line and rifabutin-based therapy.

Treiber *et al.* investigated the efficacy of a quadruple regimen containing furazolidone as a third-line therapy [54]. Administration of the quadruple regimen consisting of lansoprazole (30 mg b.i.d.), bismuth (240 mg b.i.d.), tetracycline (1 g b.i.d.) and furazolidone (200 mg b.i.d.) for 1 week yielded an eradication rate of 90% in the 10 patients.

In developing countries where resistance to metronidazole is usually very high, furazolidone in combination with tetracycline, bismuth and PPI administered for 1 week is very effective, safe and cost effective as third-line therapy, if available, for *H. pylori* eradication. However, some adverse aspects related to the use of furazolidone as a rescue therapy for *H. pylori* infection should be noted, especially regarding its potential oncogenic risk [55].

High-dose dual therapy

The increase in gastric pH induced by antisecretory drugs is crucial in order to allow antibiotics to exert their optimal activity against *H. pylori*. By increasing intragastric pH, antisecretory drugs allow the microorganism to reach the growth phase and become more sensitive to antibiotics such as amoxicillin. Moreover, PPIs exert an antibacterial action against *H. pylori*. The resistance to amoxicillin is very rare, so high-dose dual therapy might be a promising option as an alternative treatment regimen.

Miehlke *et al.* investigated the efficacy of high-dose dual therapy and quadruple therapy as salvage treatments for eradication of *H. pylori* resistant to both metronidazole and clarithromycin. Patients with at least one treatment failure and infected with *H. pylori* resistant to both metronidazole and clarithromycin were randomized to receive either omeprazole (40 mg q.i.d.) and amoxicillin (750 mg q.i.d.), or omeprazole (20 mg b.i.d.), bismuth citrate (107 mg q.i.d.), metronidazole (500 mg q.i.d.) and tetracycline (500 mg q.i.d.). Both regimens were given for 14 days. In cases with persistent infection, crossover therapy was performed. A total of 84 patients were randomized. Cure of *H. pylori* infection was achieved in 31 patients after the dual therapy and in 35 patients after the quadruple therapy (PP analysis: 83.8% [95% CI: 67.9–93.8] and 92.1% [95% CI: 78.6–98.3], respectively [$p = 0.71$]; ITT analysis: 75.6% [95% CI: 59.7–87.6] and 81.4% [95% CI: 66.6–91.6], respectively [$p = 0.60$]). This study demonstrated that both high-dose dual therapy and quadruple therapy are effective in curing *H. pylori* infection resistant to both metronidazole and clarithromycin [56]. Furthermore, Miehlke *et al.* investigated the efficacy of rifabutin-based triple therapy and high-dose dual therapy for rescue treatment of *H. pylori* [57]. Patients infected with *H. pylori* resistant to both metronidazole and clarithromycin ($n = 145$) were randomized to either esomeprazole (20 mg b.i.d.), rifabutin (150 mg b.i.d.) and amoxicillin (1 g b.i.d.) given for 7 days, or to omeprazole (40 mg t.i.d.) and amoxicillin (1 g t.i.d.) given or 14 days. The eradication rates as determined by ITT and PP analyses were 74%

(95% CI: 62.4–83.6) and 78% (95% CI: 66.7–87.3), respectively, in the rifabutin-based triple-therapy group, and 70% (95% CI: 57.5–79.7) and 75% (95% CI: 62.5–84.5), respectively, in the high-dose dual-therapy group. Premature discontinuation of treatment occurred in 2 and 5% of patients, respectively. This study demonstrated that both triple therapy with esomeprazole, rifabutin and amoxicillin, and high-dose omeprazole/amoxicillin therapy are effective and safe as rescue therapy. Therefore, high-dose dual therapy is one of the promising candidate regimens for third-line therapy. Further international study is required for this promising therapy.

Tetracycline-based therapy

Doxycycline is a widely used tetracycline antibiotic for several infections. Heep *et al.* have found no secondary resistance to doxycycline in *H. pylori* strains isolated from patients with failure of one or more eradication therapies [58].

Cammarota *et al.* investigated the efficacy of a doxycycline- and amoxicillin-based quadruple regimen administered for 1 week using a culture-guided, third-line treatment approach. Using the epsilometry test, susceptibility analysis was performed for amoxicillin, clarithromycin, metronidazole, tetracycline and levofloxacin. Patients were then treated with a culture-guided, third-line regimen: 89 patients were administered a quadruple regimen including omeprazole (20 mg b.i.d.), bismuth (120 mg b.i.d.), doxycycline (100 mg b.i.d.) and amoxicillin (1 g b.i.d.) for 1 week, and five patients were administered a triple regimen containing omeprazole, amoxicillin and levofloxacin or clarithromycin for 1 week. Overall, *H. pylori* eradication was achieved in 90% of the subjects. The quadruple regimen was effective in 81 patients (92% as determined by PP analysis and 91% as determined by ITT analysis). Four patients (80%, both as assessed by PP and ITT analysis) were *H. pylori*-negative after treatment with the triple regimen [59].

However, Akyildiz *et al.* have reported an unacceptably low eradication rate of only 36.8% following administration of a regimen consisting of ranitidine bismuth citrate–amoxicillin–doxycycline for 14 days as a first-line treatment [60]. Although a doxycycline- and amoxicillin-based therapy is a potential candidate regimen for third-line therapy, further study is needed.

Conclusion

The geographical prevalence of antimicrobial resistance should influence the choice of the first-line regimen for the treatment of *H. pylori* infection. If the clarithromycin resistance rate is below 15%, treatment can be started with a regimen based on clarithomycin. Alternatively, quadruple therapy, which has a similar success rate to a clarithromycin-based regimen, but is not limited by primary or secondary resistance to either clarithromycin or metronidazole, could be used.

After failure of second-line treatment, patients should be evaluated using a case-by-case approach, taking into account the eradication regimens attempted previously and previous

Executive summary

Bacterial resistance

- Primary resistance to clarithromycin and quinolones has been reported to range between 2 and 24%.
- Primary resistance to metonidazole has been reported to range between 8 and 80%.
  - Metronidazole resistance is much higher in developing countries than in developed countries.
- Primary resistance to amoxicillin, tetracycline and rifampins has remained low.

First-line therapy

- Recommended therapy consists of proton pump inhibitor (PPI) or ranitidine bismuth citrate with any two antibiotics of amoxicillin, clarithromycin or metronidazole given for 7–14 days.
  - Eradication failure is still seen in more than 20% of patients.
- 10-day sequential regimen is a novel, promising therapeutic approach.

Second-line therapy

- European guidelines recommend a quadruple therapy based on bismuth, tetracycline, metronidazole and PPI for a minimum of 7 days.
- A 10-day combination of levofloxacin–amoxicillin–PPI constitutes an encouraging second-line alternative.

Third-line therapy

- Eradication rates by quinolone-based therapy has been reported to range between 60 and 84%.
- A triple levofloxacin-based regimen administered for 10 days is more effective than a rifabutin-based triple regimen.
- Although tetracycline-based therapy, furazolidone-based therapy and high-dose PPI/amoxicillin therapy are potential candidates, a further study is needed.
- The choice of third-line therapy should preferably be made according to susceptibility testing.

antimicrobial therapy. The choice of third-line therapy should preferably be made according to susceptibility testing, or empirically prescribing regimens containing new antimicrobials.

Future perspective

Recommended first-line therapies are effective and well tolerated, but the widespread use over the years has led to failure of those regimens in more than 20%. Failure is mainly due to increasing antibiotic resistance, in particular against clarithromycin. Although there is an increasing trend for clarithromycin resistance, clarithromycin-based regimens remain the recommended first-line option in regions with clarithromycin resistance below 20%. Local monitoring of antimicrobial resistance needs to be guaranteed. Among novel therapeutic strategies, the sequential therapy would be promising. The further challenge could be the development of improved therapies and a vaccine.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

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