CHAPTER 3

Eradication of *Helicobacter pylori* infection favourably affects altered gastric mucosal MMP-9 levels

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Summary

Background

*Helicobacter pylori* gastritis is recognized as an important pathogenetic factor in peptic ulcer disease and gastric carcinogenesis, and is accompanied by strongly enhanced gastric mucosal MMP-9 levels.

Aim

This study was performed to investigate whether *Helicobacter pylori*-affected gastric mucosal MMP-2 and MMP-9 levels are reversible by successful treatment of the infection.

Patients and methods

Fifty-eight patients with *H. pylori*-associated gastritis were treated with a combination regimen of acid inhibitory therapy and antibiotics for 14 days. The levels and isoforms of MMP-2 and MMP-9 were measured by semi-quantitative gelatin-zymography, bioactivity assay (BIA) and enzyme-linked immunosorbent assay (ELISA) in gastric mucosal biopsy homogenates.

Results

Latent, active and total MMP-9 levels decreased consistently and significantly by successful *H. pylori* eradication, in antrum as well as corpus mucosa, compared with those prior to treatment, irrespective of the therapy regimen used. The elevated levels remained unchanged, however, when treatment failed. MMP-2 levels did not show major alterations after *H. pylori* therapy.

Conclusions

Elevated MMP-9 levels in *H. pylori*-infected gastric mucosa are reversible by eradication of the infection. No major changes in mucosal MMP-2 levels were observed by *H. pylori* eradication.
Introduction

*Helicobacter pylori* (*H. pylori*) is a curved or spiral-shaped Gram-negative bacterium that lives in the mucus layer of the gastric epithelium and also in metaplastic gastric epithelium of the esophagus or duodenum [1-3]. Infection with *H. pylori* is the most common cause of gastritis [4] and is preceded by colonization of the gastric mucosa. This infection leads to an acute gastritis that, over the course of several weeks, develops into a chronic inflammatory reaction of the mucosa [5]. Patients with long-term *H. pylori*-associated chronic gastritis are predisposed for peptic ulcer disease as well as gastric carcinoma and lymphoma [6, 7]. Matrix metalloproteinases (MMPs) are believed to play an important role in inflammation and carcinogenesis, amongst others, via the degradation and remodeling of extracellular matrix and basal membranes [8, 9]. MMPs are secreted or transmembrane endo-proteinases that share a zinc-containing catalytic domain, which is required for proteolytic activity. MMPs can degrade at least one component of the extracellular matrix. Currently, at least 25 family members have been identified which can be divided in four major subgroups, based on substrate specificity, amino acid similarity, and identifiable sequence modules: collagenases, stromelysins, gelatinases, and membrane-type MMPs. The proteins are secreted in a latent form and require extracellular activation. When activated, the enzymes are susceptible to inhibition by α2-Macroglobulin and by their antagonists, the Tissue Inhibitors of MetalloProteinases (TIMPs), by forming a complex with the (active) enzyme. This complex formation is believed to be a major regulatory mechanism [9, 10].

The gelatinases include MMP-2 or gelatinase-A, a 72 kDa proteinase, and MMP-9 or gelatinase-B, a 92 kDa proteinase, which specifically can degrade basement membrane type IV collagen, as well as gelatin, collagen type I, V, VII, X, elastin, laminin and fibronectin [11, 12]. MMP-2, an ubiquitous enzyme in normal adult tissue, is predominantly produced by stromal cells, whereas MMP-9 is predominantly produced by inflammatory cells, especially the polymorphonuclear leucocytes [9, 11, 13, 14].

In gastric biopsies from *H. pylori*-infected individuals enhanced levels of MMP-2 and MMP-9 have been described, whereas TIMP-1 and TIMP-2 levels were unaltered [15]. We previously demonstrated increased MMP-9 levels in antrum and corpus mucosa of individuals with *H. pylori*-associated gastritis, with almost unchanged MMP-2 levels, compared to *H. pylori* negative patients [16]. Furthermore, we recently reconfirmed our observation of enhanced MMP-2 and MMP-9 levels in gastric carcinoma tissues and found a consistent independent association between MMP-2 levels and patient survival [17]. As *H. pylori* gastritis is associated with gastric malignancy and *H. pylori* gastritis and gastric carcinomas are accompanied by alterations in the MMP levels we decided to investigate whether gastric mucosal MMP-2 and MMP-9 levels in *H. pylori*-induced gastritis are affected by successful eradication of the infection.
Patients, materials and methods

Patients

Biopsy specimens were collected at upper gastrointestinal endoscopy from \textit{H. pylori} positive patients between 22 and 75 years presenting with dyspeptic complaints, as described previously \cite{18, 19}. Patients who had recently used proton-pump inhibitors, corticosteroids, non-steroidal anti-inflammatory drugs (NSAIDs), bismuth compounds, sucralfate, or antibiotics were excluded. Use of low dose H$_2$-receptor antagonists was not considered to be a reason for exclusion. For histological examination, 2 biopsies were taken from the antrum, 3-5 cm proximal to the pylorus, and 2 from the corpus, 5 cm above the junction between antrum and corpus. These specimens were examined by an experienced pathologist according to the guidelines of the revised Sydney system, which provides semi-quantitative grading of histological parameters (0=normal, 1=mild, 2=moderate, 3=marked) \cite{20}. One biopsy was taken from the antrum for \textit{H. pylori} culture and processed as described previously \cite{21}. The presence of \textit{H. pylori} was assessed by a culture and/or histological identification, and confirmed by specific IgG \textit{H. pylori} antibodies. From 58 of the 63 patients included in the original study there was still biopsy material of antrum and/or corpus available for the present study to determine the MMP-2 and MMP-9 concentrations. 33 of these patients had an antral gastritis, 23 patients had a pangastritis, data of two patients were missing.

All 58 patients were treated with a combination regimen of acid-suppression and antibiotics [omeprazole 20 mg bid in 26 patients, 16 male, 10 female, mean age 53 (range 22-75) or ranitidine 150 mg bid or 300 mg qid in 32 patients, 26 male, 6 female, mean age 46 (range 22-74) with clarithromycin 500 mg tid and metronidazole 500 mg tid for 14 days, the latter only in 50\% of the omeprazole patients]. These combinations are further referred to as omeprazole and ranitidine, respectively. Successful treatment was defined as negative culture and negative histology eight weeks after the end of therapy. Four patients treated with omeprazole (double) therapy kept gastric complaints and were allowed to continue omeprazole use. They were found to be still \textit{H. pylori} positive after therapy [3 male, 1 female, mean age 39.5 (range 24-58)].

Tissue extraction and protein concentration

Homogenates were made by adding 100 µl PBST (0.05\% Tween’20 in phosphate buffered saline) per mg biopsy material and homogenizing on ice in a Potter S (B. Braun) \cite{21}. The protein concentration in the supernatant was determined by the Lowry method \cite{22}.
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**Gelatin-zymography**

The presence of active and pro forms of the matrix metalloproteinases were assessed by gelatin-zymography, as previously described [23, 24]. Ten percent polyacrylamide gels were casted in a Mini-Protean® II Dual Slab Cell (Biorad). These gels contained 1.5M Tris buffer (pH 8.8), 0.2% gelatin, 0.1% sodium dodecyl sulphate, 0.07% ammonium persulphate and 0.07% tetramethylene-diamine. First sample volumes were adjusted to obtain an equal protein content of 5 µg per sample. Two amounts (6.1 and 12.2 µg protein) of an internal standard preparation, i.e. a homogenate of a colonic carcinoma containing both MMP-2 and MMP-9, were included on each gel for correction of intergel variation and as reference for the expression in arbitrary units. After electrophoresis the gels were incubated overnight at 37°C, stained with Amido Black (0.1% amido black, 30% methanol and 10% acetic acid), and destained in a solution containing 30% methanol and 10% acetic acid. Subsequently the gels were dried between sheets of cellophane. Finally the degree of gelatin digestion was quantified by making a digital photo with a CCD Imaging System (Appligene), scanned in Aldus Photostyler 2.0 (Aldus Corporation) and analysed with Imagequant (Molecular Dynamics), using the peakfinder-mode. The gelatin digestion was reflected as a peak and the MMP levels were calculated referring to the internal standard preparations, of which the peak-height correlated highly significant with the included concentration (r=0.99, p< 0.001). The MMPs were analysed for the pro, active and total MMP levels, the latter defined as the sum of the two isoforms, and expressed as Arbitrary Units per 5 µg protein.

**Bioactivity assay**

Latent (activatable) and active MMP were also measured using a newly developed immunocapture colorimetric activity assay [17, 24]. Briefly, a polyclonal anti-MMP-2 or monoclonal anti-MMP-9 antibody (TNO-QLBR) was used as catching antibody to capture MMP-2 or MMP-9 from appropriate dilutions of the tissue homogenates, respectively 1:4 and 1:20, by overnight incubation at 4°C. Active MMP was determined directly, whereas latent MMP was activated by incubation with 0.5 mM p-aminophenylmercuric acetate for 0.5 and 2 hr at 37°C for MMP-2 and MMP-9, respectively. After washing MMP activity was assessed by adding 750 ng modified MMP-activatable pro-urokinase (Ukcol) and 0.6 mM of its chromogenic substrate S-2444 (pyro-Glu-Gly-Arg-p-nitroanilide; Chromogenix, Sweden) in assay buffer and incubating at 37°C. Reactions were performed in 96-well flat-bottomed microtitre plates, and a multichannel photometer was used to follow the absorbance kinetics at 405 nm. Results were expressed as MMP activity Units per mg protein, with Units defined as \((\Delta A_{405}/hr^2)\)*10.
ELISAs

MMP-2 and MMP-9 protein levels were measured by our highly specific ELISAs, which detected the grand total of pro-enzyme, active- and inhibitor-complexed forms of the respective MMP, as previously described [17, 24]. In brief; the same catching antibodies were used as for the bio activity assays and appropriate dilutions of tissue homogenates, respectively 1:6.7 and 1:5, were incubated overnight at 4°C. Immunodetection of MMP-9 was performed with biotinylated rabbit anti-MMP-9 and for MMP-2 using rabbit anti-MMP-2 (TNO-PG) followed by biotinylated goat anti-rabbit-IgG. After incubation with avidin/horseradish-peroxidase the chromogenic substrate 3,3',5,5'-tetramethyl benzidine and H$_2$O$_2$ were added and the reaction was stopped with H$_2$SO$_4$ and read at 405 nm. The amount of MMP was calculated from the parallel standard curves and expressed in ng MMP per mg protein.

Statistical analysis

The ELISA, zymography and BIA results are given as mean ± s.e.m. Differences between groups were evaluated for significance using the Kruskal-Wallis and Mann-Whitney U tests or the Wilcoxon Signed-Ranks test. The correlations between zymography, BIA and ELISA were assessed by the Pearson correlation procedure (SPSS for Windows 11.0 statistical package, SPSS Inc., Chicago, Illinois, U.S.A.). Differences were considered significant when $P \leq 0.05$.

Results

ELISA

Overall MMP-9 levels measured by ELISA showed a significant decrease after successful therapy in both antral and corpus mucosa (Table 1). No relevant changes in MMP-9 levels were found in the four patients with persistent \textit{H. pylori} infection, either in antrum or in corpus. The changes in the gastric MMP-9 levels were similar in the ranitidine and omeprazole treatment groups (data not shown). In addition, the levels

<table>
<thead>
<tr>
<th>Biopsy site</th>
<th>Therapy result</th>
<th>MMP-2 Before</th>
<th>MMP-2 After</th>
<th>$P$-value</th>
<th>MMP-9 Before</th>
<th>MMP-9 After</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antrum</td>
<td>Successful, $n = 49/53$</td>
<td>12.2 ± 0.7</td>
<td>10.0 ± 0.8</td>
<td>0.025</td>
<td>15.1 ± 1.7</td>
<td>2.2 ± 0.4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Unsuccessful, $n = 4$</td>
<td>15.4 ± 2.7</td>
<td>8.3 ± 1.1</td>
<td>NA</td>
<td>9.4 ± 2.7</td>
<td>12.0 ± 6.8</td>
<td>NA</td>
</tr>
<tr>
<td>Corpus</td>
<td>Successful, $n = 52/53$</td>
<td>8.0 ± 0.6</td>
<td>7.1 ± 0.7</td>
<td>NS</td>
<td>5.2 ± 0.8</td>
<td>1.5 ± 0.4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Unsuccessful, $n = 4$</td>
<td>7.4 ± 1.0</td>
<td>7.9 ± 1.8</td>
<td>NA</td>
<td>6.3 ± 2.6</td>
<td>9.5 ± 7.3</td>
<td>NA</td>
</tr>
</tbody>
</table>

Levels are expressed in ng / mg protein; NA : not applicable; NS : not significant.
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of MMP-9 in the gastric mucosa were found to be strongly related to the severity of the active inflammation. This was particularly noticeable in the corpus mucosa where the MMP-9 level in the patients with a pangastritis (7.37 ± 1.46 ng/mg protein, n=22) was significantly higher (*P*<0.02) compared to those with an antral gastritis (3.68 ± 0.84, n=27). After eradication of *H. pylori* these levels were found to be significantly decreased (*P*<0.01) in both groups but no longer significantly different between both groups (respectively, 2.56 ± 0.87 and 0.73 ± 0.19). Furthermore, the MMP-9 levels in the antrum were also found to be significantly correlated with the severity of the inflammation, as illustrated by the stepwise decrease in the MMP-9 level in accordance with the inflammation score of the combined pre- and post-treatment biopsies (Figure 1).

The MMP-2 levels showed a tendency to decrease in the antral mucosa, although the changes were relatively small, without meaningful differences between the treatment groups. MMP-2 levels were found to be unaffected in the corpus mucosa by successful eradication therapy (Table 1).

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**Figure 1.** Scatter plot of the MMP-9 levels in the antrum, as measured by ELISA, in relation to active inflammation, as scored by immunohistological evaluation, combined of biopsies from before (■) and after (□) treatment of the *H. pylori* infection. Means per inflammation score group, as indicated by the horizontal bar, were 22.9 ± 3.2 (score 2, n=11), 13.1 ± 1.9 (score 1, n=39) and 2.3 ± 0.4 ng MMP-9/mg protein (score 0, n=49). Statistical significance of the association according to the Kruskall Wallis test *P*<0.0005.
Gelatin zymography

In antral mucosa, active and latent MMP-9 levels decreased significantly after successful \textit{H. pylori} eradication, compared with before treatment (Table 2). In corpus mucosa latent MMP-9 levels decreased significantly as well, whereas active MMP-9 levels showed a non-significant decrease. The three patients with persistent \textit{H. pylori} infection also showed some decrease, though less impressive, in the active and latent MMP-9 levels after therapy. In contrast, the MMP-2 levels, active as well as latent, did not alter after therapy compared with those prior to therapy both in the \textit{H. pylori} eradicated and in the persistent \textit{H. pylori} positive group (data not shown), similar to the levels as determined by ELISA.

### Table 2 - MMP-9 levels in gastric mucosa biopsy specimens of \textit{H. pylori} positive patients before and after treatment as measured by zymography

<table>
<thead>
<tr>
<th>Biopsy site</th>
<th>Therapy result</th>
<th>Latent MMP-9</th>
<th></th>
<th></th>
<th></th>
<th>Active MMP-9</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>(P)-value</td>
<td>Before</td>
<td>After</td>
<td>(P)-value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antrum</td>
<td>Successful, (n = 34)</td>
<td>116.5 ± 17.1</td>
<td>2.2 ± 1.5</td>
<td>&lt; 0.001</td>
<td>25.7 ± 5.7</td>
<td>2.6 ± 2.3</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Unsuccessful, (n = 3)</td>
<td>102.7 ± 29.6</td>
<td>64.2 ± 46.5</td>
<td>NA</td>
<td>18.9 ± 5.1</td>
<td>7.8 ± 4.6</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Corpus</td>
<td>Successful, (n = 34)</td>
<td>25.0 ± 5.5</td>
<td>3.8 ± 1.7</td>
<td>&lt;0.001</td>
<td>3.1 ± 0.9</td>
<td>1.3 ± 0.7</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Unsuccessful, (n = 3)</td>
<td>30.3 ± 24.7</td>
<td>17.5 ± 9.5</td>
<td>NA</td>
<td>6.8 ± 6.8</td>
<td>0.7 ± 0.7</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>

Levels are expressed in AU / 5 \(\mu\)g protein homogenate; NA : not applicable; NS : not significant

Bioactivity Assay (BIA)

Latent MMP-9 levels, as assessed by the BIA, also revealed that successful treatment resulted in a significant decrease in the gastric mucosa compared with those prior to treatment, whereas no major alterations were found in the patients in whom \textit{H. pylori} was not eradicated after therapy (Table 3). With regard to the active MMP-9 levels similar results were obtained [antrum 5.6 ± 0.8 vs. 0.2 ± 0.1 (\(P<0.001\)) and corpus 2.1 ± 0.4 vs. 0.3 ± 0.1 (\(P<0.001\)), before and after successful treatment, respectively (\(n=53\)]. The changes observed in the gastric mucosal MMP-9 levels, as determined by the BIA, of the successfully \textit{H. pylori} eradicated patients again showed an identical pattern in the ranitidine and omeprazole treatment groups (data not shown). Latent MMP-2

### Table 3 - Latent MMP-9 levels in gastric mucosa biopsy specimens of \textit{H. pylori} positive patients before and after treatment as measured by BIA

<table>
<thead>
<tr>
<th>Biopsy site</th>
<th>Therapy result</th>
<th>Latent MMP-9</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>(P)-value</td>
<td></td>
</tr>
<tr>
<td>Antrum</td>
<td>Successful, (n = 47/53)</td>
<td>17.0 ± 1.8</td>
<td>1.6 ± 0.7</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Unsuccessful, (n = 4)</td>
<td>12.5 ± 4.5</td>
<td>7.3 ± 4.4</td>
<td>NA</td>
</tr>
<tr>
<td>Corpus</td>
<td>Successful, (n = 49/53)</td>
<td>5.9 ± 0.9</td>
<td>1.6 ± 0.6</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Unsuccessful, (n = 3/4)</td>
<td>3.7 ± 2.2</td>
<td>2.9 ± 1.5</td>
<td>NA</td>
</tr>
</tbody>
</table>

Levels are expressed in AU / mg protein; NA : not applicable
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levels in the gastric mucosa were once more found to be hardly affected by the H. pylori treatment regimens (data not shown). Active MMP-2 was not assessed by the BIA based on the observations in the zymography, which revealed them to be very low or absent in the gastric mucosa homogenates.

Comparison of the three techniques used for MMP-9 measurement

Positive and significant correlations of the upregulated pre-treatment MMP-9 levels in gastric mucosa of H. pylori positive individuals were found between zymography, BIA and ELISA (Table 4). After successful eradication these correlations remained significant, although the MMP-9 levels were consistently decreased. Interestingly, before therapy all MMP-9 assessments revealed a significantly higher level in the antral mucosa compared with the corpus mucosa that completely disappeared after treatment, already noticeable in Table 1. However, the correlations between the overall MMP-9 levels measured by ELISA and the MMP-9 levels measured by the gelatin-zymography or the BIA after therapy are lower than before therapy, while correlations between gelatin-zymography and BIA remain high after therapy. This observation suggests alterations in the isoform composition of MMP-9 and/or in TIMP levels.

Table 4 - Correlation of MMP-9 levels in gastric mucosa of H. pylori positive patients before and after treatment as determined by ELISA, BIA and zymography

<table>
<thead>
<tr>
<th>MMP-9</th>
<th>Assays</th>
<th>Biopsy site</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ELISA – BIA*</td>
<td>Antrum</td>
<td>0.89, &lt;0.001*</td>
<td>0.27, 0.046</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Corpus</td>
<td>0.85, &lt;0.001</td>
<td>0.44, &lt;0.001</td>
</tr>
<tr>
<td></td>
<td>ELISA – zymography*</td>
<td>Antrum</td>
<td>0.81, &lt;0.001</td>
<td>0.39, 0.018</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Corpus</td>
<td>0.65, &lt;0.001</td>
<td>0.23, NS</td>
</tr>
<tr>
<td></td>
<td>BIA – zymography*</td>
<td>Antrum</td>
<td>0.74, &lt;0.001</td>
<td>0.82, &lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Corpus</td>
<td>0.85, &lt;0.001</td>
<td>0.69, &lt;0.001</td>
</tr>
</tbody>
</table>

* 32 ≤ n ≤ 57, *Pearson correlation coefficient, P-value; NS : not significant

Discussion

H. pylori-associated chronic gastritis is recognized as a major risk factor for the development of gastric carcinoma [6, 7]. We previously showed alterations in the MMP-2 and/or MMP-9 levels in gastric tissues from patients with H. pylori-associated gastritis and from patients with gastric cancer [16, 17, 23]. In the present, uncontrolled, study we evaluated the effect of eradication therapy on these gastric MMP levels in patients with H. pylori gastritis. Latent, active and total MMP-9 levels decreased consistently and significantly after successful H. pylori eradication, in antrum as well as corpus mucosa, irrespective of the therapy regimen used. The
elevated levels remained unchanged, however, when treatment failed. The MMP-2 levels and activities in *H. pylori* positive patients did not change significantly by successful treatment.

MMP-9 in gastric mucosa is predominantly expressed by polymorphonuclear leukocytes, macrophages, (myo)fibroblasts, although *in vitro* studies also reported MMP-9 in stromal cells, inflammatory cells and epithelial cells [15, 24-27]. MMP-2 immunoreactivity was predominantly observed in epithelial cells, inflammatory cells and epithelial cells [15, 24, 25, 28]. The MMP-9 levels in the antrum of our gastritis patients were found to be two- to four-fold higher compared with the corresponding corpus, dependent on whether it was a pan- or antral gastritis. This observation corresponds very well with our previously reported observation that the active inflammatory reaction, i.e., the number of infiltrated neutrophils, in the antrum is similarly more intense compared with the corpus mucosa [19]. The higher antrum inflammation is probably caused by a slow pyloro-cardial progression of gastritis as a consequence of a less dense *H. pylori* colonization of the corpus due to local acid production [29]. The presence and activation of these inflammatory cells are caused by mucosal cytokines, e.g. TNF-α and IL-8, which are increased in *H. pylori*-induced gastritis and are also capable of inducing the production of MMP-9 and less that of MMP-2 [30, 31]. This finding can be explained by the fact that the MMP-2 encoding gene lacks an AP-1 binding site that prevents activation by TNF-α or IL-β. MMP-9, however, is an inducible matrix metalloproteinase, in contrast to MMP-2 that is expressed more constitutively [9].

With successful *H. pylori* eradication, the antigen responsible for the immune reaction is removed, leading to a slow but progressive decrease in both the active and chronic component of the gastric mucosal inflammation, including reduction of cytokine production [32-34]. In our population of patients, both forms of inflammation also decreased significantly in both antrum and corpus after successful treatment of the *H. pylori* infection [18, 19]. This decrease in inflammation was accompanied with a considerable and significant decrease of latent, active and total MMP-9, particularly in the antrum. Our results are in line with a preliminary immunohistochemical study that showed a significant decrease of enhanced MMP-9 expression in epithelial cells and fibroblasts - but not in macrophages - after *H. pylori* eradication and no alterations in MMP-9 expression where eradication failed [35]. Another immunohistochemical study, however, reported an increase in MMP-9 staining of surface mucous cells and pyloric glands of gastric antral biopsies from patients after *H. pylori* eradication [36]. The observations that the MMP-9 levels in the gastric mucosa of the unsuccessfully treated patients remain elevated suggest a direct relationship between *H. pylori* presence and MMP-9 level. Yet, in some of our assessments, e.g. zymography and BIA, some decrease in MMP-activity was noticeable in the *H. pylori* persistent patients. Probably, the acid-reducing drugs used might have an intrinsic inhibitory effect on the MMPs,
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as previously shown by the inhibitory effect of H$_2$-receptor antagonists on matrix metalloproteinases in rat gastric tissues with acetic acid-induced gastric ulcers [37, 38]. On the other hand, alterations in the level or activity of TIMPs, the endogenous MMP inhibitors, cannot be excluded but were not assessed in the present study.

Improvement and normalization of the chronic inflammatory reaction in the stomach after successful *H. pylori* eradication is accompanied by a reversal of many altered mucosal parameters that have been associated with gastric cancer and its prognosis, e.g. growth factors and cytokines [31-33], plasminogen activators [18, 39] and superoxide dismutases [19, 40]. Patients with *H. pylori*-associated chronic gastritis are predisposed for gastric carcinoma but its remains unclear whether eradication therapy also results in a reduction of gastric cancer incidence, since most of the *H. pylori* positive patients do not develop cancer, and inflammation and cancer diversity genes might play a more important role [41, 42]. Apparently also higher tissue levels of MMP-2, as in the tumors [17, 24], are required in combination with elevated MMP-9 levels for the development of *H. pylori* gastritis to carcinoma. Our study is not conclusive in that respect due to the absence of major alterations in the MMP-2 levels. Larger studies, including pathogenicity classification of the *H. pylori* strains, are needed to get a better insight into the relevance of changes in the MMP expression in the development of gastric cancer. In addition, genetic susceptibility might also play a role, as illustrated by the MMP-7 $^{181A>G}$ gene polymorphisms which has recently been found to be associated with both gastric ulcerogenesis in *H. pylori* infection and gastric cancer, which provides a potential genetic link and implicates other MMPs in the association between both disorders [43, 44].

In conclusion, the *H. pylori*-associated increased MMP-9 levels in antrum and corpus mucosa decrease significantly by successful eradication of *H. pylori*. No major changes occurred in the MMP-2 levels and activities by eradication therapy and in the MMP-9 levels when eradication failed.

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