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Concentrations of α- and β-defensins in gastric juice of patients with various gastroduodenal diseases

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Abstract

AIM: To determine the concentration of α- and β-defensins in gastric juice of patients with various gastroduodenal diseases.

METHODS: Concentrations of human neutrophil peptides (HNPs) 1-3, the major forms of α-defensins, and human β-defensin (HBD)-1 and HBD-2 were measured by radioimmunoassay in plasma and gastric juice of 84 subjects, consisting of 54 *Helicobacter pylori*-infected and 30 uninfected subjects. They included 33 patients with chronic gastritis (CG), 12 with gastric ulcer (GU), 11 with duodenal ulcer (DU), 11 with benign gastric polyp (BGP) and 16 with normal mucosa (N group) on upper endoscopy. Plasma pepsinogen I and II levels, biomarkers for gastric mucosal inflammation and atrophy, were also measured.

RESULTS: Gastric juice HNPs 1-3 levels in patients with CG, GU and BGP were significantly higher than those in patients with DU and N. Gastric juice HBD-2 concentrations in patients with CG and GU were significantly higher than those in the N group, but were significantly lower in DU patients than in GU patients. Gastric juice HBD-1 levels and plasma levels of these peptides were similar in the patient groups. Concentrations of gastric juice HNPs 1-3 and HBD-2 of in *H pylori*-infected patients were significantly different from those in uninfected subjects. HNPs 1-3 concentrations in gastric juice correlated negatively with plasma pepsinogen I levels and I/II ratios. HBD-2 levels in gastric juice correlated positively and negatively with plasma pepsinogen II concentrations and I/II ratios, respectively.

CONCLUSION: HNPs 1-3 and HBD-2 levels in gastric juice are diverse among various gastrointestinal diseases, reflecting the inflammatory and atrophic events of the background gastric mucosa affected by *H pylori*.

Key words: *Helicobacter pylori* infection; Gastroduodenal diseases; α-defensins; β-defensin

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INTRODUCTION

Recently, endogenous antimicrobial peptides have been identified as key elements of innate host defence against infection[1-5]. Defensin, a single chain cationic peptide with a molecular weight ranging from 3 000 to 4 500, is one of the most extensively studied classes of such naturally occurring antibiotics[1-3]. It exhibits a wide variety of microbicidal activities against Gram-positive and -negative bacteria, mycobacteria, fungi and certain enveloped viruses[1-3]. Human defensins is divided into α- and β-defensins, based on the arrangements of three intramolecular disulphide bridges[1-3]. Among six members of human α-defensin identified so far, human neutrophil peptides 1-4 (HNP-1, HNP-2, HNP-3 and HNP-4) are localized in azurophilic granules of neutrophils. HNPs 1-3 are very similar and different only in a single N-terminal amino acid, whereas HNP-4 shares only 32% amino acid sequences homologous to HNPs 1-3[1-3]. Human defensin-5 (HD-5) and HD-6, are present in intestinal Paneth's cells[1,2]. On the other hand, the four human β-defensins including human β-defensin (HBD)-1 and HBD-2 are primarily produced by epithelia at mucosal sites[1-3]. In fact, *in vivo* studies have shown elevated concentrations of these defensins in blood and body fluids from patients infected with various microorganisms[1-3].

Helicobacter pylori (*H pylori*) is the major cause of chronic gastritis and peptic ulcer disease. Long-persisting infection leads to atrophic gastritis, which increases the risk of gastric cancer[6-10]. Gastric hyperplastic polyp is also known to be associated with this organism[11]. Several studies showed the constitutive expression of HBD-1 and induced expression of HBD-2 upon *H pylori* infection both *in vitro* and *in vivo*[12-16]. In addition, a significant difference was found in gastric mucosal expression of HD-6 with respect to *H pylori* status[16]. This data emphasizes the significance of defensins in bacterial infection. However, there is no information on the secretion of defensins into the gastric lumen and their concentrations in gastric juice either in *H pylori*-related or unrelated conditions.

We have developed a sensitive, specific radioimmunoassay (RIA) for HNPs 1-3 and HBDs 1-2, major forms of α- and β-defensins respectively[17,18]. Employing this assay system, we measured their gastric juice concentrations in patients with various gastroduodenal disorders, with special reference to *H pylori* infection. In addition, we assessed the relationship between the concentrations of α- and β-defensins and

circulating levels of pepsinogen I, pepsinogen II, and gastrin, which were evaluated as biomarkers for precancerous lesions, especially chronic atrophic gastritis and peptic ulcer[19,20].

MATERIALS AND METHODS

Patients and sampling

A total of 84 patients referred for diagnostic upper gastrointestinal endoscopy between September 2002 and August 2003 were enrolled in the present study. The following exclusion criteria were applied for enrolment in the study, including the use of non-steroidal anti-inflammatory drugs, proton pump inhibitors, histamine H_2-receptor antagonists or antibiotics within 4 wk prior to the present study, and history of severe concomitant diseases, upper gastrointestinal surgery or gastric cancer. On the day of endoscopy, blood samples were withdrawn, transferred into tubes containing ethylenediaminotetraacetic acid (EDTA)-2Na and aprotinin, centrifuged. Plasma was separated and stored at -80 ℃ until assay.

At the beginning of endoscopy (XQ 200; Olympus Optical Co., Tokyo, Japan), a sample of gastric juice was aspirated into a collection tube containing EDTA-2Na and aprotinin using an aspiration instrument (PW-6P-1, Olympus) under endoscopic guidance. Gastric juice samples were immediately neutralized to pH 7.0 with 1N NaOH and frozen at -80 ℃ until measurement[21]. Each biopsy specimen was endoscopically obtained from both the antrum within 2 cm of the pyloric ring and the middle portion of the corpus along the greater curvature, and used for rapid urease test (Helicocheck, Otsuka Pharmaceutical Co., Tokushima, Japan).

Circulating anti-H pylori antibody and gastrin and pepsinogen concentrations

Plasma anti-*H pylori* immunoglobulin (Ig) G antibody was assessed using an enzyme linked immunosorbent assay kit (HEL-p TEST, AMRAD Co., Melbourne, Victoria, Australia). The cut-off value was determined according to the protocol provided by the manufacturer. Plasma gastrin and pepsinogen concentrations were determined by radioimmunoassay with commercial kits (Double Antibody Gastrin kit, DPC, Los Angeles, CA, and PEPSINOGEN I/II-RIABEAD kit, Dainabot, Tokyo, respectively).

Measurement of HNPs 1-3 and HBD-1 and -2 levels in plasma and gastric juice

Concentrations of α- and β-defensins in plasma and gastric juice were measured by RIA established in our laboratory[17,18]. Briefly, full-length HNP-1, HBD-1 and HBD-2 were synthesized using a peptide synthesizer (model 430, Applied Biosystems, Foster City, CA) and purified by reverse phase high performance liquid chromatography (RP-HPLC). The synthetic peptides were used for immunizing New Zealand white rabbits by multiple intracutaneous and subcutaneous injections. They were radioiodinated and the ^{125}I-labeled peptides were purified by RP-HPLC on a TSK ODS 120A column (Tosoh Co., Tokyo). A diluted sample or standard peptide solution (100 μL) was incubated for 24 h with each 100 μL antiserum diluent. The final dilutions were 1:21 000, 1:460 000 and 1:4 200 000 for HNP-1, HBD-1 and HBD-2 respectively. The ^{125}I-labeled solution (16 000 cpm in 100 μL) was added and the mixture was incubated again for another 24 h. Normal rabbit serum and anti-rabbit IgG goat serum were then added and stored for 16 h. Bound and free ligands were separated by centrifugation. All procedures were performed at 4 ℃ and duplicate assays were carried out. Each 10 μL of plasma and gastric juice was used to determine the levels of defensins. The antiserum for HNP-1 recognized HNP-2 and HNP-3 equally on a molar basis, and thus the RIA data was expressed as the sum of HNPs 1-3[18]. The intra-assay and inter-assay coefficients of variation were <10% in all RIA analyses[17,18].

Statistical analysis and ethical considerations

Statistical analyses were performed using Fisher's exact, χ^2, Student's *t*, Mann-Whitney U, Kruskal-Wallis, Spearman rank and Wilcoxon signed rank tests, as appropriate. A *P* value less than 0.05 was accepted as statistically significant. Data were expressed as mean±SD.

All examinations were conducted according to the Good Clinical Practice and the Declaration of Helsinki, and approved by the University Ethics Committee. All samples were obtained with written informed consent of the patients prior to their inclusion in the study. All experiments involving animals were approved by the Ethics Review Committees for Animal Experimentation of the participating universities.

RESULTS

Patient demographics

The study population consisted of 33 patients with chronic gastritis (CG), 12 with gastric ulcer (GU), 11 with duodenal ulcer (DU), 11 with benign gastric polyp (BGP) and 16 with normal mucosa of the upper gastrointestinal tract at endoscopy (N group). They included 40 men and 44 women, with a mean age of 54 years (range 28-80 years). There were no significant differences among the groups in background data on age, sex, body mass index, current tobacco use and alcohol intake.

Concentrations of defensins in gastric juice and plasma in various gastroduodenal diseases

The HNPs 1-3 levels in gastric juice significantly differed among patients with diverse gastroduodenal diseases (Figure 1A). Compared to the N group, patients with CG, GU and BGP had significantly higher HNPs 1-3 concentrations in gastric juice ($P<0.05$, each). The gastric juice HNPs 1-3 levels in DU patients were significantly lower than those in CG ($P<0.01$), GU and BGP ($P<0.05$, each) patients. There were no significant differences in plasma HNPs 1-3 concentrations among the disease groups (Table 1).

Gastric HBD-2 concentrations juice in CG and GU patients were significantly higher than those in the N group (Figure 1B, $P<0.05$, each). There was a significant difference in gastric juice HBD-2 levels between GU and DU patients ($P<0.05$). Plasma HBD-2 levels did not differ among the groups (Table 1).

With respect to HBD-1, there were no significant differences among the groups both in gastric juice (Figure 1C) and in plasma concentrations (Table 1).

Table 1 Plasma human neutrophil peptides 1-3, β-defensin-2 and β-defensin-1 levels in patients with various gastroduodenal diseases (mean±SD)

	Human neutrophil peptides 1-3 (ng/mL)	β-defensin 2 (pg/mL)	β-defensin-1 (ng/mL)
Chronic gastritis	520.8±40.0	250.4±84.1	8.68±1.08
Gastric ulcer	512.1±75.3	225.3±32.6	8.81±1.26
Duodenal ulcer	560.4±121.9	269.8±46.5	8.37±1.14
Benign gastric polyp	606.4±99.5	206.2±70.0	7.81±1.32
Normal mucosa	552.7±69.5	196.3±55.4	7.08±1.88

H pylori status and concentrations of defensins in gastric juice and plasma

Of the 33 patients with CG, 27 (82%) had *H pylori* infection. Patients with GU and DU were all infected with the organism. Histopathologically, the BGP group consisted of 5 patients with hyperplastic polyps and 6 fundic gland polyps, the positive rate of the infection was 80% (4/5) and 0% (0/6) respectively. All N group subjects were negative for the infection. The overall prevalence of *H pylori* infection in the study population was 64% (54/84).

Gastric juice HNPs 1-3 and HBD-2 levels in *H pylori*-infected CG and BGP patients tended to be higher than those in uninfected ones, but the differences were statistically insignificant (Figures 2A, B). The overall gastric juice HNPs 1-3 and HBD-2 concentrations in *H pylori*-infected patients were significantly higher than those in uninfected ones (Figures 2A, B; $P<0.01$ and $P<0.05$ respectively).

There was no significant difference in HBD-1 levels in gastric juice of patients with gastroduodenal infections (Figure 2C). *H pylori* status had no significant impact on plasma concentrations of defensins (Table 2).

Table 2 Plasma human neutrophil peptides 1-3, β-defensin-2 and β-defensin-1 levels and *H pylori* status (mean±SD)

	H pylori-infected	*H pylori*-uninfected
Human neutrophil peptides 1-3 (ng/mL)	544.2±36.4	518.3±53.1
β-defensin 2 (pg/mL)	286.5±55.8	238.5±53.4
β-defensin 1 (ng/mL)	7.66±0.94	8.13±0.71

Plasma gastrin and pepsinogen levels in patients with various gastroduodenal diseases (Table 3)

Plasma pepsinogen I concentrations were significantly higher in patients with DU than in those with CG and BGP, and subjects of the control N group ($P<0.005$ for CG and N, and $P<0.05$ for BGP). Plasma pepsinogen II concentrations in patients with GU were significantly higher than those in patients with CG, DU, and BGP, and subjects of the N group ($P<0.005$, $P<0.01$, $P<0.05$ and $P<0.0001$ respectively). Plasma pepsinogen II levels were significantly higher in patients with CG than in subjects of the N group ($P<0.0001$). The pepsinogen I/II ratios in patients with GU and CG were also significantly lower than those in patients with DU and subjects of the N group (GU, $P<0.0005$ and $P<0.0001$, respectively, and CG, $P<0.05$ and $P<0.0005$, respectively). Plasma gastrin concentrations in patients with CG, GU and DU were significantly higher than those in subjects of the N group ($P<0.01$ for CG and $P<0.05$ for GU and DU).

Table 3 Plasma pepsinogen I and II levels, pepsinogen I/II ratio and gastrin concentrations in patients with various gastroduodenal diseases (mean±SD)

	Pepsinogen I (ng/mL)	Pepsinogen II (ng/mL)	Pepsinogen I/II ratio	Gastrin (pg/mL)
Chronic gastritis	52.0±26.5	19.5±9.8	3.3±1.9	84.9±41.3
Gastric ulcer	67.1±31.2	31.4±12.5	2.4±1.3	69.3±35.9
Duodenal ulcer	76.7±20.2	18.1±8.9	4.5±0.8	69.2±34.6
Benign gastric polyp	49.0±29.1	17.3±16.9	4.0±2.8	57.1±30.4
Normal mucosa	46.7±26.2	9.2±5.4	5.2±1.5	48.5±22.1

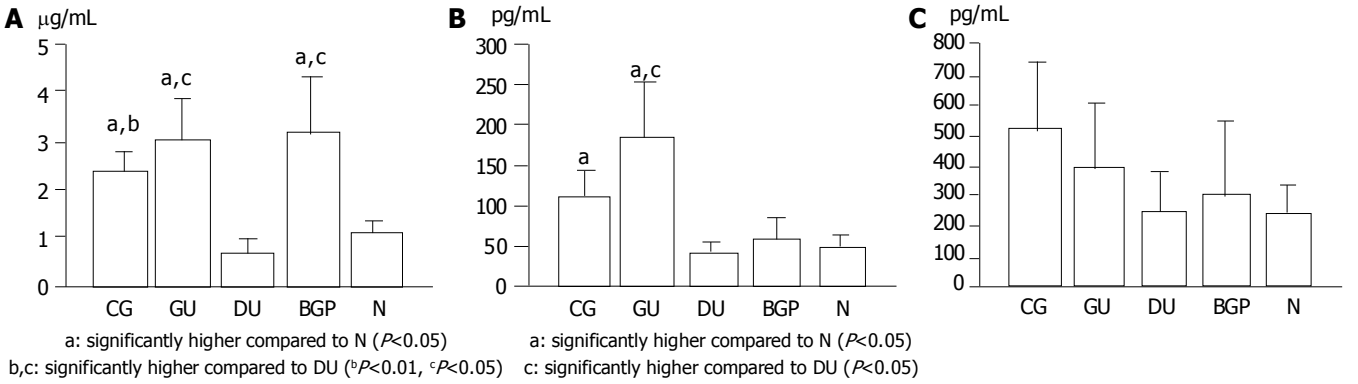

Figure 1 Concentrations of human neutrophil peptides 1-3 (A), human β-defensin 2 (B), and human β-defensin 1 (C) in gastric juice of patients with diverse gastroduodenal diseases. CG: chronic gastritis; GU: gastric ulcer; DU: duodenal ulcer; BGP: benign gastric polyp; N: normal mucosa in upper gastrointestinal tract.

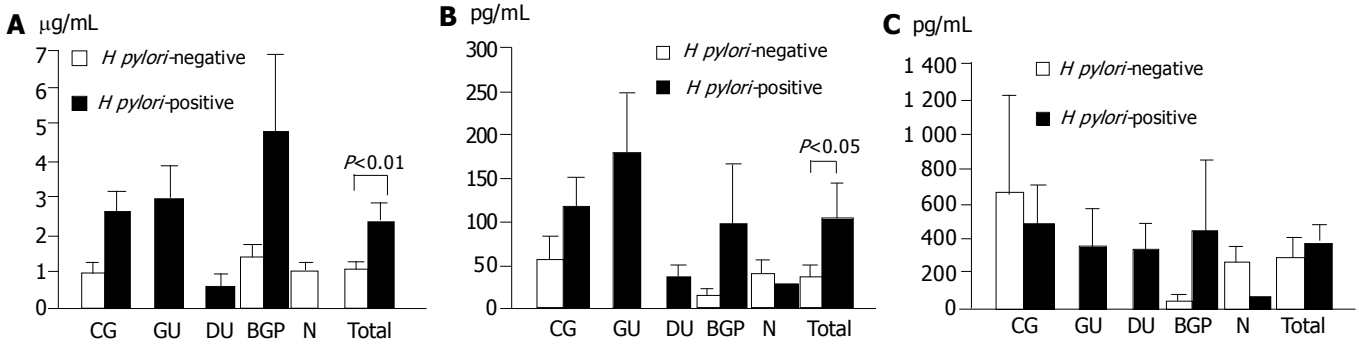

Figure 2 Concentrations of human neutrophil peptides 1-3 (A), human β-defensin 2 (B) and human β-defensin 1 (C) in gastric juice of *Helicobacter pylori*-infected and uninfected subjects.

Table 4 Plasma pepsinogen I and II levels, pepsinogen I/II ratio and gastrin concentrations with respect to *H pylori* status

	Pepsinogen I (ng/mL)	Pepsinogen II (ng/mL)	Pepsinogen I/II ratio	Gastrin (pg/mL)
H pylori-infected	62.9±25.6	23.5±11.4	3.1±2.5	76.1±39.7
H pylori-uninfected	42.5±23.2	8.6±4.9	5.2±3.4	56.2±27.7

H pylori status significantly influenced plasma pepsinogen II concentrations (21.9±8.7 ng/mL and 8.6±7.0 in *H pylori*-infected and uninfected patients respectively, $P<0.005$) and I/II ratio (2.8±2.5 and 5.3±4.7 in *H pylori*-infected and uninfected patients respectively, $P<0.005$) in CG patients. For the whole group, there were significant differences in plasma pepsinogen I and II concentrations, I/II ratios and circulating gastrin levels with respect to *H pylori* infection (Table 4, $P<0.001$, $P<0.0001$, $P<0.0001$ and $P<0.05$, respectively).

Correlation between defensin levels and plasma gastrin and pepsinogen concentrations

Concentrations of HNPs 1-3 in gastric juice correlated negatively and significantly with plasma pepsinogen I concentrations and I/II ratio (Table 5, $P<0.005$ and $P<0.01$) respectively. Concentrations of HBD-2 in gastric juice correlated positively with plasma pepsinogen II concentrations and negatively with I/II ratio (Table 5, $P<0.05$, each). There were no significant correlations between each level of defensins in gastric juice and plasma gastrin concentrations. Concentrations of HBD-1 in gastric juice and those of antimicrobial peptides in plasma did not correlate with plasma levels of any of the above biomarkers.

Table 5 Correlation coefficients between each parameter

	Pepsinogen I	Pepsinogen II	Pepsinogen I/II	Gastrin
Human neutrophil peptides 1-3	-0.492	0.043	-0.418	-0.265
β-defensin 2	0.073	0.297	-0.321	0.087
β-defensin 1	-0.114	0.023	-0.175	0.140

DISCUSSION

Our sensitive RIA system allows the determination of α- and β-defensin concentrations in gastric juice, as well as in other body fluids and blood[17,18,22,23]. Thus, in the present study, we demonstrated that the concentrations of HNPs 1-3 and HBD-2 in gastric juice were significantly different in patients with various gastroduodenal diseases. Gastric juice HNPs 1-3 concentrations in patients with BGP, GU and CG were significantly higher than those in subjects of the N group, suggesting a pathophysiological role of HNPs in these diseases. The same might be true for the association of HBD-2 with GU and CG, as its levels in gastric juice of patients with the two diseases were significantly elevated compared to those of N subjects. DU patients had rather low concentrations of HNPs 1-3 and HBD-2 in gastric juice, which were equivalent to each level of subjects with the endoscopically normal mucosa. On the other hand, there were no significant differences in plasma concentrations of defensins assessed among diverse conditions. These results suggest that measurement of HNPs 1-3 and HBD-2 concentrations in gastric juice, but not in plasma, can be suggestive of the presence of gastroduodenal lesions or allows distinguishing patients with gastric ulcers from those with duodenal ulcers.

Recent studies have shown the inducible expression of HBD-2 messenger ribonucleic acid (mRNA) in response to *H pylori* infection in cultured gastric epithelial cells[12-14]. In clinical setting of gastritis, mRNA and peptide expression of HBD-2 and HD-6 is evidently increased in gastric mucosa infected with the organism[15,16]. There are several *in vitro* observations of the secretion of HBD-2 peptide upon *H pylori* infection[14,24]. In keeping with these data, we noted significantly higher gastric juice HNPs 1-3 and HBD-2 concentrations in *H pylori*-infected subjects compared to each level of uninfected subjects. Since the infection is closely associated with chronic gastritis, gastric ulcer and hyperplastic polyp[6-8,25], as confirmed in the present study, the elevated levels of HNPs 1-3 and HBD-2 in such diseases might be in part attributable to the augmented production/ release caused by *H pylori* infection. However, this mechanism cannot solely explain their reduced levels in DU patients, infected with the organism.

We found that concentrations of HNPs 1-3 and HBD-2 in gastric juice correlated with plasma pepsinogens. HNPs 1-3 levels correlated negatively and significantly with plasma pepsinogen I level and I/II ratio, whereas HBD-2 levels correlated positively with pepsinogen II and negatively with I/II ratio respectively. In line with these results, we observed significantly high pepsinogen II levels and low I/II ratios in GU and CG patients. In BGP patients, a trend similar to CG was noted for pepsinogens. In contrast, pepsinogen I levels were rather high in DU patients, linking to the low values of HNPs 1-3 in this condition. In many clinico-epidemiological studies, the diagnostic potential of circulating profiles of pepsinogens in predicting the topography and severity of gastric mucosal inflammation and atrophy has been established[19,20,26,27]. Therefore, we believe that concentrations of these defensins in gastric juice are different in diverse gastroduodenal diseases, reflecting the inflammatory and atrophic events of the background gastric mucosa, mostly affected by persistent *H pylori* infection in our series[28-30].

Our results showed no significant differences in HBD-1 concentrations in gastric juice as well as in plasma among diverse gastroduodenal diseases, providing further evidence for its constitutive nature[1-3,12]. Since ingestion of contaminated food or water exposes the gastric mucosa to a wide variety of microbial pathogens, the constitutive expression of HBD-1 plays a 'surveillance-like' role during normal homeostasis of human stomach[31].

In conclusion, evident diversities were found in gastric juice concentrations of HNPs 1-3 and HBD-2 in patients with various gastroduodenal diseases. There were significant differences in HNPS 1-3 and HBD-2 levels with respect to *H pylori* status and significant correlations with plasma pepsinogens, biomarkers for the severity and extent of gastric mucosal inflammation and atrophy. It is suggested that the inflammatory and atrophic events of the background gastric mucosa, caused by *H pylori* infection, could explain the differences in the concentrations of these antimicrobial peptides among diverse gastroduodenal conditions.

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