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RETROSPECTIVE COHORT STUDY

Membrane-bound mucins and mucin terminal glycans expression in idiopathic or *Helicobacter pylori*, NSAID associated peptic ulcers

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Abstract

AIM: To determine the expression of membrane-bound mucins and glycan side chain sialic acids in *Helicobacter pylori* (*H. pylori*)-associated, non-steroidal inflammatory drug (NSAID)-associated and idiopathic-gastric ulcers.

METHODS: We studied a cohort of randomly selected patients with *H. pylori* (group 1, n = 30), NSAID (group 2, n = 18), combined *H. pylori* and NSAID associated gastric ulcers (group 3, n = 24), and patients with idiopathic gastric ulcers (group 4, n = 20). Immunohistochemistry for MUC1, MUC4, MUC17, and staining for *Erythrina cristagalli* agglutinin and *Sambucus nigra* agglutinin (SNA) lectins was performed on sections from the ulcer margins.

RESULTS: Staining intensity of MUC17 was higher in *H. pylori* ulcers (group 1) than in idiopathic ulcers (group 4), 11.05 ± 3.67 *vs* 6.93 ± 4.00 for foveola cells, and 10.29 ± 4.67 *vs* 8.00 ± 3.48 for gland cells, respectively (P < 0.0001). In contrast, MUC1 expression was higher in group 4 compared group 1, 9.89 ± 4.17 *vs* 2.93 ± 5.13 in foveola cells and 7.63 ± 4.60 *vs* 2.57± 4.50 for glands, respectively (P < 0.0001). SNA lectin staining was increased in group 4, in parallel to elevated MUC1 expression, indicating more abundant α2-6 sialylation in that group.

CONCLUSION: Cytoplasmic MUC17 staining was sig-

nificantly decreased in the cases with idiopathic ulcer. The opposite was observed for both MUC1 and SNA lectin. This observation may reflect important pathogenic mechanisms, since different mucins with altered sialylation patterns may differ in their protection efficiency against acid and pepsin.

Key words: Mucin; Idiopathic ulcer; *Helicobacter pylori*; Glycosylation; Peptic ulcer disease; Mucosal cytoprotection

Core tip: Peptic ulcers are diverse in origin, and the proportion of idiopathic gastric ulcers not related to *Helicobacter pylori* infection or non-steroidal inflammatory drug therapy is increasing. Membrane-bound mucin proteins in peptic ulcer has not been described previously, and are important for understanding of ulcer pathogenesis and subsequently treatment. Major findings of this paper include the observation that MUC17 staining was significantly decreased in idiopathic ulcer cases, whereas MUC1 mucin expression was increased. Idiopathic ulcers also demonstrated higher sialic acid mucin residue staining. These alterations in mucin core proteins and sialylation patterns may be associated with differences in epithelial cytoprotection, and therefore may play a role in ulcer pathogenesis.

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INTRODUCTION

The pathogenesis of the majority of peptic ulcers is related to infection with *Helicobacter pylori* (*H. pylori*) or to the use of aspirin or non-steroidal inflammatory drug (NSAID)-type medications. A growing number of patients have ulcers not associated with these or other causes, termed idiopathic ulcers. Idiopathic ulcers have been shown to have unique clinical characteristics and are associated with higher morbidity and mortality[1-7].

Mucins, heavily glycosylated high-molecular-weight glycoproteins, are expressed at high levels in gastric epithelial cells and help form the gastric mucous unstirred layer that protects the mucosa from acid and pepsin. There are 2 groups of mucins encoded by 21 genes: secreted and membrane-bound[8-10]. Alterations of gastric mucins may play a primary or secondary role in the pathogenesis of peptic ulcer disease[2,11]. In a previous study of patient cohort with gastric ulcer we did not observe any significant changes in secretory mucin expression (MUC5AC and MUC6) between various pathogenic types of ulcer (idiopathic, *H. pylori*-associated, or aspirin/NSAID-associated ulcer)[1].

The pattern of membrane-bound mucins and sialic acid expression in peptic ulcer disease has never been examined. The membrane-bound mucins MUC1, MUC4 and MUC17 have been previously described as part of the normal glycocalyx and forms the mucin barrier of the stomach[12,13]. MUC1 is a ubiquitous epithelial cell surface mucin that exhibits altered expression and glycosylation during inflammation and neoplastic development. It has been shown to be important for limiting the inflammation and attachment of *H. pylori* in gastric mucosa[14]. MUC4 is a trans-membrane mucin whose expression is altered in gastrointestinal neoplasia[15]. MUC17 has been shown to inhibit apoptosis, promote intestinal epithelial migration, and to be cytoprotective in animal models of colitis[16-18]. In addition, peripheral glycan residues on mucin glycoproteins have been shown to play a role in mucosal barrier function and bacterial populations[19].

Since membrane-bound mucins may be a significant factor in gastric epithelial cytoprotection, we choose to study the expression pattern of gastric membrane-bound mucins in various types of peptic ulcer. Comparisons of the relative expression of these cytoprotective proteins in idiopathic *vs* other ulcer types may help elucidate pathogenic factors unique to this type of ulcer.

MATERIALS AND METHODS

Patients

The study cohort was described in details in our previous paper examining secreted-mucins, MUC5AC and MUC6, in the mucosa of gastric ulcer patients[1]. In the present study we examined specimens from the same patients for the expression of membrane-bound mucins and peripheral sialic acid side-chains. As described previously, after approval of the protocol by the IRB of Rabin Medical Center, consecutive patients with gastric ulcers who underwent endoscopy and gastric biopsy were included and stratified according to etiology: *H. pylori*-associated (group 1), NSAID-associated (group 2), both *H. pylori*- and NSAID-associated (group 3), or no etiologic factor (idiopathic, group 4). Patients with aspirin exposure were included in the NSAID groups. Cases where no biopsies could be found, with proven malignancy, or with a specific gastrointestinal disease diagnoses other then peptic ulcer, were excluded[1]. As described previously, standard clinical practice was used for *H. pylori* diagnosis for this study, which used standard clinical criteria based on both histology using hematoxylin and eosin staining and rapid urease testing. If available, breath tests for *H. pylori* diagnosis were also used[1]. Special stains such as Giemsa and toluidine blue could be used at the discretion of the pathologist. One positive test was required for the diagnosis of *H. pylori* infection, and two negative tests (*e.g.*, histology and rapid urease testing) was required for a patient to be categorized as negative.

Tissue samples and mucin immunohistochemistry

We used the same methods as described previously[1]. In brief: paraffin embedded blocks was cut into 4 μm thick sections, deparaffinized in xylene and rehydrated using a graded ethanol series. Antigen was retrieved by boiling the slides in a microwave oven for 15 min in 0.01 mol/L citrate buffers (pH = 6.0). Endogenous peroxidase was blocked with a 3% H_2O_2-methanol solution, and the slides were incubated in 10% normal goat serum for 30 min to prevent nonspecific staining. The tissue sections were incubated overnight at 4 ℃ with primary antibody. The standard anti-mouse Ig HRP-DAB and biotin-streptavidin-peroxidase methods were then used, and the sections were lightly counterstained with hematoxylin. Histologically normal gastric biopsies were used as positive controls for MUC1, MUC4 and MUC17. The sections incubated with phosphate-buffered saline (0.01 mol/L, pH = 7.4) instead of primary antibody were used as negative controls. Primary antibodies included anti-MUC1 used at 1:100 dilution, (Santa Cruz Biotechnology, Santa Cruz, CA); monoclonal anti-MUC4 clone 8G7, used as described previously[20]; and anti-MUC17 polyclonal antibody against a synthetic peptide corresponding to a portion of the MUC17 tandem repeat sequence (PTTAEGTSMPTSTPSE) was used as described previously[21].

Sambucus nigra agglutinin and Erythrina cristagalli Agglutinin fluorescent and histochemical staining

The lectins studied included *Sambucus nigra* agglutinin (SNA), to detect sialic acid in α2-6 glycosidic linkage to underlying glycans, most commonly galactose[22]; and *Erythrina cristagalli* agglutinin, or *Erythrina cristagalli* agglutinin (ECA), to detect *N*-acetyllactosamine (Galβ1-4GlcNAc)[23], the most common glycan structure underlying sialic acids in α2-6 linkage. *N*-acetyllactosamine is exposed when the sialic acid is removed. For fluorescent staining the tissues were blocked with 1% BSA/PBS (bovine serum albumin, Sigma-Aldrich) for 10 min, and incubated with fluorescein-conjugated *Sambucus nigra* agglutinin (SNA-FITC, 1:1000 dilution, Vector Labs) in HEPES/NaCl buffer (10 mmol/L HEPES, 150 mmol/L NaCl pH 7.5) for 1 h at room temperature. The nuclei were counterstained with DAPI, and tissues were mounted with aqueous mounting medium (Vector Labs).

For lectin histochemical staining, endogenous peroxidase was first blocked with H_2O_2, and endogenous biotin was blocked with Avidin-Biotin blocking kit (Vector labs) according to manufacturer's instructions. Tissues were further blocked with 1% BSA/TBST (0.05 mol/L Tris HCl, 150 mmol/L NaCl pH = 8.0, 0.1% Tween 20) for 10 min, and incubated for 30 min with biotinylated-SNA (1:1000 dilution, Vector Labs) or with biotinylated-ECA (1:2500 dilution, Vector labs) in 1% BSA/TBST supplemented with 10 mmol/L $CaCl_2$ and 10 mmol/L $MnCl_2$ at room temperature. Tissues were washed and incubated for 30 min with horseradish peroxidase conjugated streptavidin (Streptavidin-HRP, 1:500 dilution, Jackson Immunoresearch), followed by 5 min incubation with Vector Blue substrate (Vector Labs) in 0.1 mol/L Tris/levimasole. Nuclei were counterstained with nuclear fast red for 30 min, and tissues were mounted with aqueous mounting medium (Vector Labs).

Staining Interpretation

All slides were scanned by the NanoZoomer and digitalized (NanoZoomer 2.0 series, Hamamatsu, Japan). Whole slide scans allow complete review, examination and analysis of all parts of the tissue, accurately assigned identical low or high power microscopy fields. Cytoplasm staining was assessed in at least 10 high-power fields by two observers at 2 sites, the foveola and the glands. Range of cytoplasmic staining included 0: 0%; 1: < 10%; 2: 11%-25%; 3: 26%-50%; 4: 51%-75%; 5: > 75%. Intensity of staining included 0: no staining; 1: weak staining; 2: intermediate staining; 3: Strong staining. The averages of the grades were calculated and the final staining score was defined as the product of scores for the range and intensity of cytoplasmic staining, as described previously[1]. All specimens were scored blinded to clinical data.

Statistical analysis

We performed statistical analysis using Statistical Package for the Social Sciences software 19.0 (SPSS, Inc.). Patient groups were compared using the Pearson χ^2 test, Fisher's exact test and Duncan test. *P* values were considered significant when $\leqslant 0.05$.

RESULTS

Patient characteristics

Patient characteristics and clinical data were detailed previously[1]. In brief: the study group included a total of 92 patients; Group 1: *H. pylori*-associated gastric ulcer (n = 30), Group 2: aspirin and/or NSAID-associated gastric ulcer (n = 18), Group 3: combined *H. pylori* and NSAID-associated gastric ulcer (n = 24), and Group 4: *H. pylori*/NSAID negative or idiopathic gastric ulcer (n = 20). The average age was 66.6 ± 15.5 years (range 18-95 years), with 47.8% women. Group 4 patients (idiopathic ulcer) were predominantly hospitalized inpatients compared with the other groups (80% *vs* 45.8%, P = 0.007). In addition, mortality was greater in idiopathic ulcer patients compared with other groups (25% *vs* 9.7%, P = 0.04). No significant differences between the groups were found in origin, primary indication for endoscopy, haemoglobin level, ulcer number, size or location within the stomach[1].

Mucin staining scores

MUC1 protein was strongly expressed on the apical membrane of the glands and mucosal surface foveola epithelial cells. The staining score for the surface epithelium was generally higher than gland cells but did not reach significance (Table 1). MUC1 expression was significantly higher for both superficial foveola epithelium and glands, in patients with *H. pylori*/NSAID negative

Table 1 MUC1, MUC4 and MUC17 mucin and lectin expression scores

Patient group	MUC1		MUC4		MUC17		SNA		ECA	
	Surface foveola cell	Gland cell	Surface foveola cell	Gland cell	Surface foveola cell	Gland cell	Surface foveola cell	Gland cell	Surface foveola cell	Gland cell
H. pylori+/NSAID-	2.93 ± 5.13	2.57 ± 4.50	2.79 ± 4.22	5.50 ± 3.14	11.05 ± 3.65	10.29 ± 4.67	2.00 ± 2.63	2.69 ± 4.01	13.27 ± 3.08	5.62 ± 4.37
	n = 28	*n* = 28	*n* = 14	*n* = 14	*n* = 21	*n* = 21	*n* = 16	*n* = 16	*n* = 26	*n* = 26
H. pylori-/NSAID+	0.00[a] ± 0	0.00 ± 0	3.38 ± 3.07	3.54 ± 3.82	9.23 ± 3.64	9.57 ± 4.73	5.06 ± 5.52	3.44 ± 4.70	14.07 ± 2.01	4.47 ± 4.32
	n= 14	*n* = 14	*n* = 13	*n* = 13	*n* = 20	*n* = 20	*n* = 18	*n* = 18	*n* = 15	*n* = 15
H. pylori+/NSAID+	11.00 ± 3.78	7.33 ± 4.13	3.75 ± 2.93	6.44 ± 5.17	5.93 ± 2.99	8.80 ± 3.76	4.18 ± 5.57	6.94 ± 5.30	13.78 ± 2.73	4.61 ± 4.64
	n = 18	*n* = 18	*n* = 16	*n* = 16	*n* =19	*n* = 19	*n* = 17	*n* = 17	*n* = 18	*n* = 18
H. pylori-/NSAID-	9.89 ± 4.17	7.63 ± 4.60	4.59 ± 3.77	4.00 ± 4.12	6.93 ± 4.00	8.00 ± 3.48	6.17 ± 4.69	3.94 ± 5.45	13.06 ± 4.06	8.00 ± 5.15
	n = 19	*n* = 19	*n* = 17	*n* = 17	*n* =22	*n* = 22	*n* = 18	*n* = 18	*n* = 18	*n* = 18

[a]$P = 0.04$, $P < 0.0001$, $P < 0.0001$ between Group 2 to Group 1, 3, and 4, respectively. ECA: *Erythrina cristagalli* agglutinin; *H. pylori*: *Helicobacter pylori*; NSAID: Non-steroidal anti-inflammatory drug; SNA: *Sambucus nigra* agglutinin.

(idiopathic) ulcers (Group 4) than in *H. pylori* positive/NSAID negative (Group 1, $P < 0.0001$) (Figure 1, Table 2). MUC1 was not expressed in ulcers with *H. pylori* negative/NSAID positive status (Group 2), and this finding was statistically significant when compared to the other 3 groups. MUC4 expression was not significantly different between the glands and surface epithelium, nor between Group 1 (*H. pylori* positive/NSAID negative ulcers) and patients with Group 4 (idiopathic ulcer) patients (Figure 1A, Tables 1 and 2). MUC17 protein was strongly expressed on the apical membrane of the mucosal epithelial cells. MUC17 was also expressed in small vacuoles within the foveola surface cells (Figure 1B). The staining intensity was similar between the foveola and glands (Figure 1, Table 1). Staining score was higher in Group 1 (*H. pylori* positive/NSAID negative) than in Group 4 (idiopathic ulcer) patients, with mean score of 11.05 ± 3.67 *vs* 6.93 ± 4.00 for foveola, and 10.29 ± 4.67 *vs* 8.00 ± 3.48 for gland cells, respectively ($P < 0.0001$). In contrast, the opposite was observed with MUC1, with higher MUC1 expression in idiopathic ulcers (Group 4) compared with *H. pylori* positive/NSAID negative ulcers (Group 1) (Figure 1, Table 2).

Sialic acid staining score

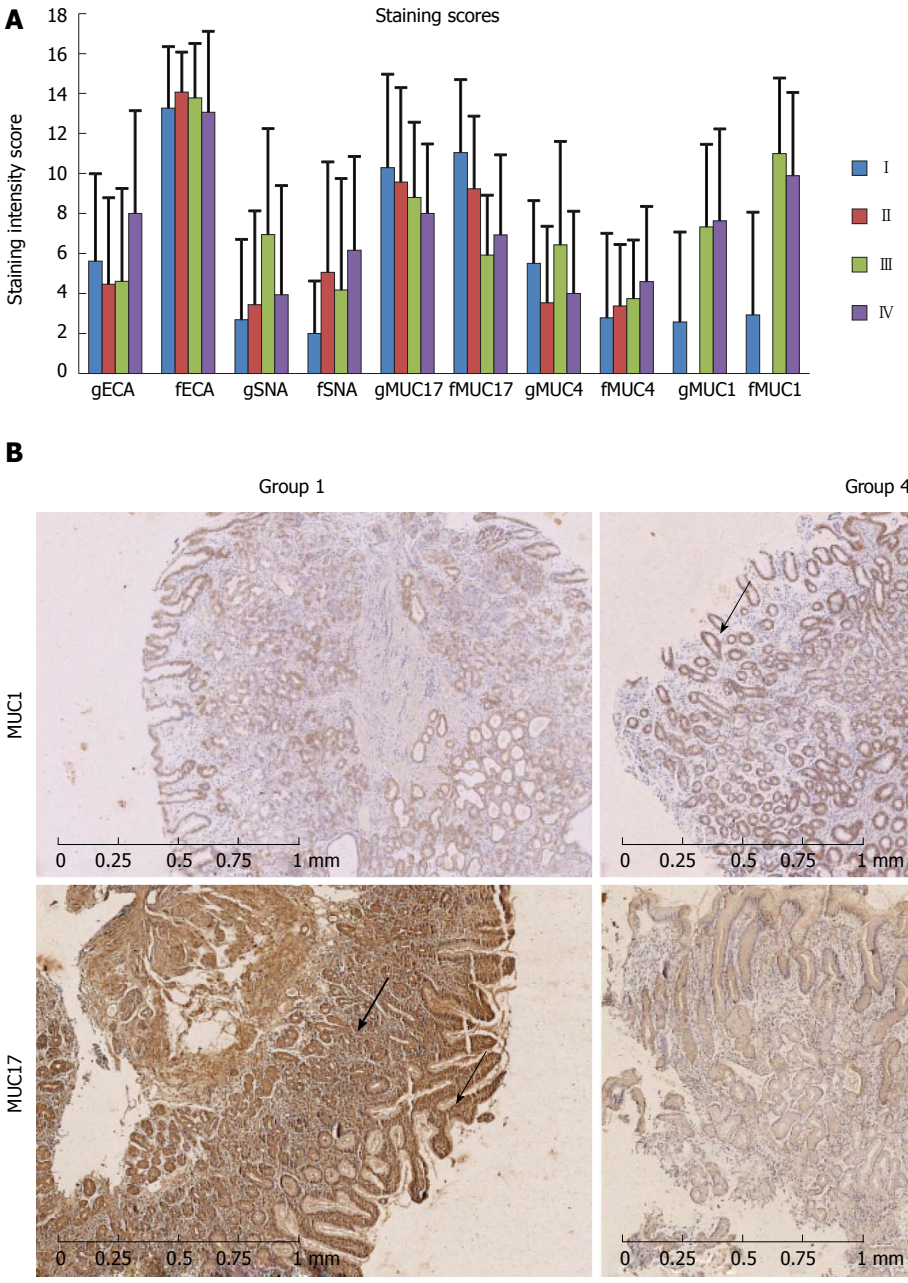
The expression of α2-6 linked sialic acid residues, as stained by SNA lectin, was the same in the surface foveola or the glands (Table 1). Staining intensity was lower in *H. pylori* positive/NSAID negative patients (Group 1) than in patients with *H. pylori*/NSAID negative ulcers (Group 4) only in the surface epithelium ($P = 0.004$) (Figure 1A and C, Table 2). MUC1 and SNA have similar staining behaviour when Group 1 (*H. pylori* positive/NSAID negative) and Group 4 (*H. pylori*/NSAID negative ulcers) patients are compared. Both have higher staining score in the foveola and glands of patients with *H. pylori*/NSAID negative ulcers than patients with *H. pylori* positive/NSAID negative ulcers, reaching significance for both MUC1 and SNA in the foveola ($P < 0.0001$, and $P = 0.004$, respectively), but only for MUC1 in the glands ($P < 0.0001$ and $P = 0.457$, respectively). In general ECA staining intensity was significantly higher in the surface foveola epithelium than in the glands in all groups ($P < 0.0001$). No significant difference in ECA was found between ulcers from Groups 1 and 4 patients in the gland or surface epithelium staining score (Figure 1A, Table 2).

DISCUSSION

Peptic ulcers arise from a variety of pathogenic pathways and differ in their clinical characteristics[24]. We have observed that patients with *H. pylori*/NSAID-negative ulcers had multiple co-morbidities, were more often inpatients at the time of endoscopy, had fewer subacute presentations, and had poorer survival[1]. This concurs with Chan *et al*[3], who noted that three quarters of patients with acutely bleeding *H. pylori*/NSAID-negative ulcers have significant co-morbidity including major organ failure and malignancy.

Gastric membrane-bound mucin expression has not been previously studied in the setting of peptic ulcer disease. In the present study, the distribution of immunohistochemical staining for MUC1, MUC4 and MUC17, and lectin binding to representative glycan residues in the margins of gastric peptic ulcer was studied. We compared the staining intensity between 4 patient groups: *H. pylori* positive, NSAID positive, either positive or both negative. The MUC1 gene has 1201 nucleotides, is located on chromosome 1q21, and has a short intracellular domain, a transmembrane domain, and a large glycosylated extracellular domain[10,25]. The other membrane-bound mucins such as MUC4 and MUC17 have general structural similarity, but with the addition of extracellular cysteine-rich EGF-like domains[10]. The EGF-like domains of MUC17 have been shown to inhibit intestinal cell apoptosis and stimulate cell migration, contributing to cell restitution[17]. The functions of MUC4 and MUC17 in normal gastric mucosa are unknown.

In the present study, cytoplasmic MUC17 staining associated with *H. pylori* infection was significantly increased, and was higher at the surface (foveola) and glands areas than in the cases with idiopathic ulcer. The opposite was demonstrated for MUC1 that significantly increased in the foveola and glands in the group of idiopathic ulcer patients. This observation of MUC1 up regulation might be important, since the protection


A
Staining scores
Staining intensity score
18
16
14
12
10
8
6
4
2
0
gECA
fECA
gSNA
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gMUC17
fMUC17
gMUC4
fMUC4
gMUC1
fMUC1
I
II
III
IV
B
Group 1
Group 4
MUC1
MUC17
0 0.25 0.5 0.75 1 mm
C
Group 1: H. pylori+ Aspirin-
Sialidase treated
SNA-FITC

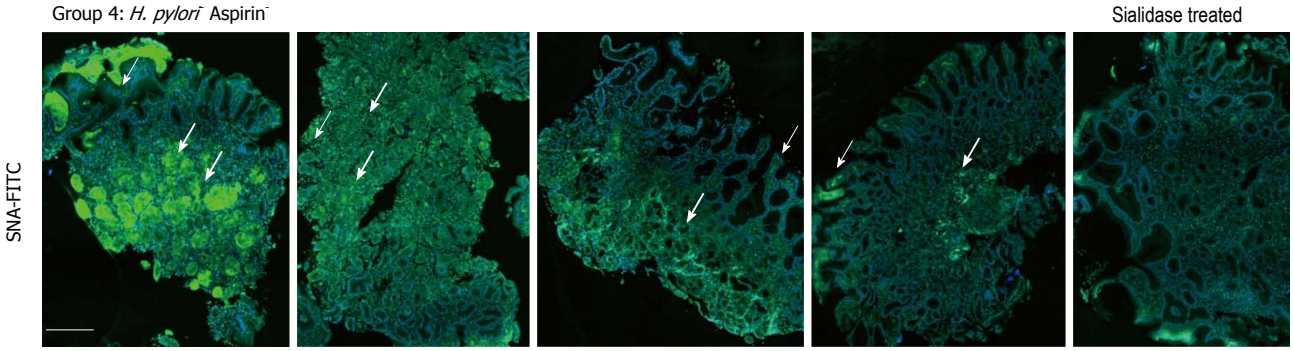

Figure 1 Membrane bound mucin and lectin expression in different gastric ulcer groups. A: Staining scores in the different gastric ulcer groups (mean ± SD). Ⅰ = group 1 = *H. pylori* positive/NSAID negative; Ⅱ = group 2 = *H. pylori* negative/NSAID positive; Ⅲ = group 3 = *H. pylori* positive/NSAID positive; Ⅳ = group 4 = *H. pylori* positive/NSAID negative (f = foveola, g = gland). Statistical significance was determined by two-tail *T* test: SNA foveola group Ⅰ *vs* Ⅳ ($P < 0.05$), MUC1 foveola and gland group Ⅰ *vs* Ⅳ ($P < 0.0001$), MUC17 foveola and gland group Ⅰ *vs* Ⅳ ($P < 0.0001$); B: MUC1 stain is stronger in group 4 compared to group 1 in both gland (thick arrows) and foveola cells (thin arrows), in contrast, MUC17 stain is stronger in group 1 compared to group 4 in foveola (thin arrows) and to a lesser extent in gland cells (thick arrows). Scale bars = 1 mm; C: SNA-FITC lectin staining of sialic acid in α2-6 glycosidic linkage in four representative gastric ulcer samples from Group 1 (top panels) and Group 4 (lower panels). SNA-FITC staining (green color) is stronger in group 4 compared to group 1 in foveola cells (thin arrows) and in gland cells (thick arrows). No staining is observed in sialidase treated tissue, confirming SNA binding specificity. Scale bars = 250 μm. NSAID: Non-steroidal anti-inflammatory drug; SNA: *Sambucus nigra* agglutinin; *H. pylori*: *Helicobacter pylori*.

efficiency against acid, pepsin and bacteria provided by different mucins is probably not equal. The decrease of MUC17 expression in the idiopathic ulcer group, even though partially compensated by higher expression of MUC1, may be insufficient for induction of effective protection. We also found a significant decrease in MUC1 expression in *H. pylori* negative/NSAID positive ulcers. This finding was statistically significant when compared to the other 3 groups, and cannot easily be explained. We speculate that NSAID therapy decreased MUC1 expression through decrease of prostaglandin E synthesis. The presence of *H. pylori* infection may mask this phenomenon through other causes of mucin synthesis and secretion. Interestingly, foveolar expression of sialic acids in α2-6 glycosidic linkage was significantly higher in these cases, a finding that cannot be attributed towards a specific mucin, but may be a global phenomenon that particularly belongs to idiopathic ulcer disease. The increase in sialic acid residues may affect mucins' protection against aggressive luminal agents or bacteria[26,27]. The distance between adjacent carbohydrates side-chains may increase due to the negative charge of sialic acid at the end of each chain, leading to increased exposure of the mucin backbone to acid and pepsin.

The interactions of pathogenic factors such as aspirin, NSAIDs and *H. pylori* with gastric mucins is complex. Aspirin and NSAIDs inhibit mucosal cyclooxygenase, which is responsible for homeostatic mechanisms including gastric mucin secretion and accumulation[28]. *H. pylori* disrupts the assembly of the mucin molecule via inhibition of galactosyltransferase responsible for synthesis of mucin *O*-glycans[29,30]. Furthermore, *H. pylori* reduces gastric mucous viscosity by elevating pH through urease secretion, thereby enhancing its motility within gastric mucous[31]. Kobayashi *et al*[32] demonstrated how BabA and SabA adhesins on *H. pylori* bind to Lewis B and sialyl Lewis X (a tetrasaccharide) blood group antigens on MUC5AC, facilitating colonization. On the other hand, gastric mucins have antimicrobial properties which are directed against *H.pylori*. Kawakubo *et al*[33] demonstrated that unique *O*-glycans in MUC6 inhibit bacterial biosynthesis of cholesteryl-α-D-glucopyranoside, a major cell wall component. Linden *et al*[34] suggest that mucins decorated with Lewis B (the binding site for the *H. pylori* BabA adhesin) effectively bind *H. pylori* thereby impairing its colonization of the mucosal surface. In addition, mice deficient in Muc1 demonstrate increased *H. pylori* attachment and gastric inflammation[14,35]. Muc1 has been shown to be shed from surface gastric cells and may act as a decoy to limit bacterial attachment to the cell surface[35]. It is conceivable that other membrane bound mucins protect gastrointestinal cell surfaces by the same mechanism.

A limitation of our study is the retrospective nature of the data and sample collection, which precluded further classification of the specimens. We used routine histologic methods with special stains if needed for diagnosis of *H. pylori* infection, which has been shown to be highly accurate in clinical settings[36], along with rapid urease testing and breath tests, however; we did not use specific immunostaining methods for *H. pylori* diagnosis. A prospective study is necessary for performing multiple tests for *H. pylori* and assaying serum salicylate and plasma thromboxane in order to eliminate false negative tests for *H. pylori* (due to PPI, bismuth or antibiotics), and cases of surreptitious or unreported NSAID use. In addition, we only compared expression of mucins at ulcer margin among the groups, and did not compare expression in other areas of the stomach. Therefore we could not determine if the mucin expression observed is localized and related to secondary changes or is more widespread.

In conclusion, expression patterns of membrane-bound mucins in *H. pylori*/NSAID-negative or idiopathic ulcers are unique, and need to be further examined in

Table 2 Comparison of mucin and sugar residues staining score between group 1 and group 4

Mucin/sugar	Group 1	*n*	Group 4	*n*	*P* value
MUC1 foveola	2.93 ± 5.13	28	9.89 ± 4.17	19	< 0.0001
MUC1 glands	2.57 ± 4.50	28	7.63 ± 4.60	19	< 0.0001
MUC4 foveola	2.79 ± 4.22	14	4.59 ± 3.77	17	0.221
MUC4 glands	5.50 ± 3.14	14	4.00 ± 4.12	17	0.286
MUC17 foveola	11.05 ± 3.65	21	6.93 ± 4.00	22	< 0.0001
MUC17 glands	10.29 ± 4.67	21	8.00 ± 3.48	22	< 0.0001
SNA foveola	2.00 ± 2.63	16	6.17 ± 4.69	18	0.004
SNA glands	2.69 ± 4.01	16	3.94 ± 5.45	18	0.457
ECA foveola	13.27 ± 3.08	26	13.06 ± 4.06	18	0.846
ECA glands	5.62 ± 4.37	26	8.00 ± 5.15	18	0.107

ECA: *Erythrina cristagalli* agglutinin; SNA: *Sambucus nigra* agglutinin.

prospective well controlled studies. Idiopathic peptic ulcers are an increasingly encountered entity, with unique clinical and endoscopic features. Future efforts should focus on identifying genetic and epigenetic factors which regulate mucin secretion and mucin glycan modifications in this setting, especially related to MUC1 and MUC17 membrane-bound mucins.

COMMENTS

Background

Peptic ulcers arise from a variety of pathogenic pathways and differ in their clinical characteristics. Mucin-type proteins are cytoprotective proteins that are highly expressed in the gastrointestinal tract, and are grouped in secreted and membrane-bound groups. The different types of membrane-bound mucins and glycosides that are associated with different types of peptic ulcer have not previously been studied.

Research frontiers

Membrane-bound mucins include MUC1, MUC3, MUC4, and MUC17 and differ in terms of structure and biologic properties. Differences in mucin expression may reflect different pathophysiological pathways of peptic ulcer subtypes.

Innovations and breakthroughs

This is the first description of membrane bound mucins in different types of peptic ulcer. Major findings included that cytoplasmic MUC17 staining was significantly decreased in the cases with idiopathic ulcer, whereas the opposite was observed for both MUC1 and SNA lectin.

Applications

The study results suggest that different types of peptic ulcer may be associated with different membrane bound mucin patterns. Further studies are needed to determine if this is a cause or effect of the specific pathology, and whether determination of gastric mucin expression can help predict susceptibility to specific types of ulcer. In addition, further studies are needed to determine if manipulation or augmentation of specific gastric mucins may prevent ulcer formation or improve healing.

Peer review

In this search the authors reported significant variations of mucin levels in different ulcers and support rationally the results. The search appears of clinical and speculative interest. Although the study is retrospective on small sample of patients, it is able to lead significant speculations about mucosal damage in course of *Helicobacter pylori* infection and non-steroidal inflammatory drug intake.

REFERENCES

1 **Boltin D**, Halpern M, Levi Z, Vilkin A, Morgenstern S, Ho SB, Niv Y. Gastric mucin expression in Helicobacter pylori-related, nonsteroidal anti-inflammatory drug-related and idiopathic ulcers. *World J Gastroenterol* 2012; **18**: 4597-4603 [PMID: 22969235 DOI: 10.3748/wjg.v18.i33.4597]

2 **Niv Y**. H. pylori/NSAID--negative peptic ulcer--the mucin theory. *Med Hypotheses* 2010; **75**: 433-435 [PMID: 20444554 DOI: 10.1016/j.mehy.2010.04.015]

3 **Chan HL**, Wu JC, Chan FK, Choi CL, Ching JY, Lee YT, Leung WK, Lau JY, Chung SC, Sung JJ. Is non-Helicobacter pylori, non-NSAID peptic ulcer a common cause of upper GI bleeding? A prospective study of 977 patients. *Gastrointest Endosc* 2001; **53**: 438-442 [PMID: 11275883 DOI: 10.1067/mge.2001.112840]

4 **Xia HH**, Wong BC, Wong KW, Wong SY, Wong WM, Lai KC, Hu WH, Chan CK, Lam SK. Clinical and endoscopic characteristics of non-Helicobacter pylori, non-NSAID duodenal ulcers: a long-term prospective study. *Aliment Pharmacol Ther* 2001; **15**: 1875-1882 [PMID: 11736717 DOI: 10.1046/j.1365-2036.2001.01115.x]

5 **Wong GL**, Wong VW, Chan Y, Ching JY, Au K, Hui AJ, Lai LH, Chow DK, Siu DK, Lui YN, Wu JC, To KF, Hung LC, Chan HL, Sung JJ, Chan FK. High incidence of mortality and recurrent bleeding in patients with Helicobacter pylori-negative idiopathic bleeding ulcers. *Gastroenterology* 2009; **137**: 525-531 [PMID: 19445937 DOI: 10.1053/j.gastro.2009.05.006]

6 **Gisbert JP**, Calvet X. Review article: Helicobacter pylori-negative duodenal ulcer disease. *Aliment Pharmacol Ther* 2009; **30**: 791-815 [PMID: 19706147 DOI: 10.1111/j.1365-2036.2009.04105.x]

7 **McColl KE**. How I manage H. pylori-negative, NSAID/aspirin-negative peptic ulcers. *Am J Gastroenterol* 2009; **104**: 190-193 [PMID: 19098868 DOI: 10.1038/ajg.2008.11]

8 **Kim YS**, Ho SB. Intestinal goblet cells and mucins in health and disease: recent insights and progress. *Curr Gastroenterol Rep* 2010; **12**: 319-330 [PMID: 20703838 DOI: 10.1007/s11894-010-0131-2]

9 **Bafna S**, Kaur S, Batra SK. Membrane-bound mucins: the mechanistic basis for alterations in the growth and survival of cancer cells. *Oncogene* 2010; **29**: 2893-2904 [PMID: 20348949 DOI: 10.1038/onc.2010.87]

10 **Hattrup CL**, Gendler SJ. Structure and function of the cell surface (tethered) mucins. *Annu Rev Physiol* 2008; **70**: 431-457 [PMID: 17850209 DOI: 10.1146/annurev.physiol.70.113006.100659]

11 **Niv Y**, Boltin D. Secreted and membrane-bound mucins and idiopathic peptic ulcer disease. *Digestion* 2012; **86**: 258-263 [PMID: 23075498 DOI: 10.1159/000341423]

12 **Gum JR**, Crawley SC, Hicks JW, Szymkowski DE, Kim YS. MUC17, a novel membrane-tethered mucin. *Biochem Biophys Res Commun* 2002; **291**: 466-475 [PMID: 11855812 DOI: 10.1006/bbrc.2002.6475]

13 **Ho SB**, Shekels LL, Toribara NW, Kim YS, Lyftogt C, Cherwitz DL, Niehans GA. Mucin gene expression in normal, preneoplastic, and neoplastic human gastric epithelium. *Cancer Res* 1995; **55**: 2681-2690 [PMID: 7780985]

14 **McGuckin MA**, Every AL, Skene CD, Linden SK, Chionh YT, Swierczak A, McAuley J, Harbour S, Kaparakis M, Ferrero R, Sutton P. Muc1 mucin limits both Helicobacter pylori colonization of the murine gastric mucosa and associated gastritis. *Gastroenterology* 2007; **133**: 1210-1218 [PMID: 17919495 DOI: 10.1053/j.gastro.2007.07.003]

15 **Senapati S**, Chaturvedi P, Sharma P, Venkatraman G, Meza JL, El-Rifai W, Roy HK, Batra SK. Deregulation of MUC4 in gastric adenocarcinoma: potential pathobiological implication in poorly differentiated non-signet ring cell type gastric cancer. *Br J Cancer* 2008; **99**: 949-956 [PMID: 18781152 DOI: 10.1038/sj.bjc.6604632]

16 **Ho SB**, Dvorak LA, Moor RE, Jacobson AC, Frey MR, Corredor J, Polk DB, Shekels LL. Cysteine-rich domains of muc3 intestinal mucin promote cell migration, inhibit apoptosis, and accelerate wound healing. *Gastroenterology* 2006; **131**: 1501-1517 [PMID: 17101324 DOI: 10.1053/j.gastro.2006.09.006]

17 **Luu Y**, Junker W, Rachagani S, Das S, Batra SK, Heinrikson RL, Shekels LL, Ho SB. Human intestinal MUC17 mucin augments intestinal cell restitution and enhances healing of experimental colitis. *Int J Biochem Cell Biol* 2010; **42**: 996-1006

[PMID: 20211273 DOI: 10.1016/j.biocel.2010.03.001]
18 **Ho SB**, Luu Y, Shekels LL, Batra SK, Kandarian B, Evans DB, Zaworski PG, Wolfe CL, Heinrikson RL. Activity of recombinant cysteine-rich domain proteins derived from the membrane-bound MUC17/Muc3 family mucins. *Biochim Biophys Acta* 2010; **1800**: 629-638 [PMID: 20332014 DOI: 10.1016/j.bbagen.2010.03.010]
19 **Bergstrom KS**, Xia L. Mucin-type O-glycans and their roles in intestinal homeostasis. *Glycobiology* 2013; **23**: 1026-1037 [PMID: 23752712 DOI: 10.1093/glycob/cwt045]
20 **Swartz MJ**, Batra SK, Varshney GC, Hollingsworth MA, Yeo CJ, Cameron JL, Wilentz RE, Hruban RH, Argani P. MUC4 expression increases progressively in pancreatic intraepithelial neoplasia. *Am J Clin Pathol* 2002; **117**: 791-796 [PMID: 12090430 DOI: 10.1309/7Y7N-M1WM-R0YK-M2VA]
21 **Moniaux N**, Junker WM, Singh AP, Jones AM, Batra SK. Characterization of human mucin MUC17. Complete coding sequence and organization. *J Biol Chem* 2006; **281**: 23676-23685 [PMID: 16737958 DOI: 10.1074/jbc.M600302200]
22 **Shibuya N**, Goldstein IJ, Broekaert WF, Nsimba-Lubaki M, Peeters B, Peumans WJ. The elderberry (Sambucus nigra L.) bark lectin recognizes the Neu5Ac(alpha 2-6)Gal/GalNAc sequence. *J Biol Chem* 1987; **262**: 1596-1601 [PMID: 3805045]
23 **De Boeck H**, Loontiens FG, Lis H, Sharon N. Binding of simple carbohydrates and some N-acetyllactosamine-containing oligosaccharides to Erythrina cristagalli agglutinin as followed with a fluorescent indicator ligand. *Arch Biochem Biophys* 1984; **234**: 297-304 [PMID: 6548353 DOI: 10.1016/0003-9861(84)90352-7]
24 **Soll AH**, Vakil NB. Peptic ulcer disease: genetic, environmental, and psychological risk factors and pathogenesis. In: Feldman M, editor. UpToDate. Alphen aan den Rijn: Wolters Kluwer, 2013
25 **Niv Y**. MUC1 and colorectal cancer pathophysiology considerations. *World J Gastroenterol* 2008; **14**: 2139-2141 [PMID: 18407586 DOI: 10.3748/wjg.14.2139]
26 **Fuster MM**, Esko JD. The sweet and sour of cancer: glycans as novel therapeutic targets. *Nat Rev Cancer* 2005; **5**: 526-542 [PMID: 16069816 DOI: 10.1038/nrc1649]
27 **Severi E**, Hood DW, Thomas GH. Sialic acid utilization by bacterial pathogens. *Microbiology* 2007; **153**: 2817-2822 [PMID: 17768226 DOI: 10.1099/mic.0.2007/009480-0]
28 **Phillipson M**, Johansson ME, Henriksnäs J, Petersson J, Gendler SJ, Sandler S, Persson AE, Hansson GC, Holm L. The gastric mucus layers: constituents and regulation of accumulation. *Am J Physiol Gastrointest Liver Physiol* 2008; **295**: G806-G812 [PMID: 18719000 DOI: 10.1152/ajpgi.90252.2008]
29 **Tsukashita S**, Kushima R, Bamba M, Sugihara H, Hattori T. MUC gene expression and histogenesis of adenocarcinoma of the stomach. *Int J Cancer* 2001; **94**: 166-170 [PMID: 11668493 DOI: 10.1002/ijc.1460]
30 **Tanaka S**, Mizuno M, Maga T, Yoshinaga F, Tomoda J, Nasu J, Okada H, Yokota K, Oguma K, Shiratori Y, Tsuji T. H. pylori decreases gastric mucin synthesis via inhibition of galactosyltransferase. *Hepatogastroenterology* 2003; **50**: 1739-1742 [PMID: 14571831]
31 **Celli JP**, Turner BS, Afdhal NH, Keates S, Ghiran I, Kelly CP, Ewoldt RH, McKinley GH, So P, Erramilli S, Bansil R. Helicobacter pylori moves through mucus by reducing mucin viscoelasticity. *Proc Natl Acad Sci USA* 2009; **106**: 14321-14326 [PMID: 19706518 DOI: 10.1073/pnas.0903438106]
32 **Kobayashi M**, Lee H, Nakayama J, Fukuda M. Roles of gastric mucin-type O-glycans in the pathogenesis of Helicobacter pylori infection. *Glycobiology* 2009; **19**: 453-461 [PMID: 19150806 DOI: 10.1093/glycob/cwp004]
33 **Kawakubo M**, Ito Y, Okimura Y, Kobayashi M, Sakura K, Kasama S, Fukuda MN, Fukuda M, Katsuyama T, Nakayama J. Natural antibiotic function of a human gastric mucin against Helicobacter pylori infection. *Science* 2004; **305**: 1003-1006 [PMID: 15310903 DOI: 10.1126/science.1099250]
34 **Lindén S**, Semino-Mora C, Liu H, Rick J, Dubois A. Role of mucin Lewis status in resistance to Helicobacter pylori infection in pediatric patients. *Helicobacter* 2010; **15**: 251-258 [PMID: 20633185 DOI: 10.1111/j.1523-5378.2010.00765.x]
35 **McGuckin MA**, Lindén SK, Sutton P, Florin TH. Mucin dynamics and enteric pathogens. *Nat Rev Microbiol* 2011; **9**: 265-278 [PMID: 21407243 DOI: 10.1038/nrmicro2538]
36 **Wright CL**, Kelly JK. The use of routine special stains for upper gastrointestinal biopsies. *Am J Surg Pathol* 2006; **30**: 357-361 [PMID: 16538056]

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