Brief Communications

Tissue transglutaminase activity in human gastric mucosa according to *Helicobacter pylori* infection

Maria Pina Dore[1,2], Giovanni Mario Pes[1], Alessandra Errigo[1], Alessandra Manca[1] and Giuseppe Realdi[3]

[1]Dipartimento di Scienze Mediche, Chirurgiche e Sperimentali, University of Sassari, Sassari 07100, Italy; [2]Baylor College of Medicine, Department of Medicine, Gastroenterology Section, Houston, TX 77030, USA; [3]Department of Medicine, University of Padova, Padova 35121, Italy

Corresponding author: Maria Pina Dore. Email: mpdore@uniss.it

Impact statement

Tissue transglutaminase (t-TG) is unique among TG enzymes because of its additional role in several physiological and pathological activities, including inflammation, fibrosis, and wound healing. The presence of t-TG has previously been described in the intestine of human and animal models, yet studies on t-TG activity in human gastric mucosa are missing. *Helicobacter pylori* infection is the major cause of gastritis and peptic ulcers. For the first time, our results show that t-TG activity was significantly higher in antral specimens of patients with chronic active gastritis associated with *H. pylori* infection compared to *H. pylori* negative chronic gastritis and normal antral mucosa. These findings suggest that t-TG has a role in the natural history of human gastritis, which requires further investigation but may be an avenue for new therapeutic options.

Abstract

Tissue transglutaminase (t-TG) is a multifunctional protein involved in the healing of gastric erosions and ulcers in animal models. The aim of this study was to measure gastric t-TG activity in patients with dyspepsia according to *Helicobacter pylori* infection and cytotoxin-associated gene A (cagA) and vacuolating cytotoxin (vacA) subtype status. Patients undergoing upper endoscopy not taking any medications were enrolled. Tissue-TG activity was determined in homogenates of antral specimens using a radiometric assay and was expressed in pmol/mg. The cagA and vacA genotypes were determined by PCR amplification using gene-specific oligoprimers. Data from 46 patients were available (17 of them were positive for *H. pylori*). Antral t-TG activity was significantly increased in *H. pylori* positive patients compared to *H. pylori* negative patients (6437 ± 3691 vs. 3773 ± 1530 pmol/mg; $P = 0.001$) according to Mann–Whitney U test. Patients with *H. pylori* negative gastritis had higher t-TG activity than patients with normal gastric mucosa. The specimens infected with cagA positive strains (72%) displayed greater t-TG activity than cagA negative samples (7358 ± 4318 vs. 4895 ± 1062 pmol/mg; $P = 0.237$). Similarly, t-TG activity was higher in *H. pylori* vacA*s1/m1* strains vs. vacA *s1/m2* (7429 vs. 5045 pmol/mg; $P = 0.744$), and vacA *s1/m1* vs. *s2/m2* (7429 vs. 4489 pmol/mg; $P = 0.651$) but the results were not significant. No differences were found between histology, endoscopy features and t-TG activity. These results show that t-TG activity is significantly greater in gastritis associated with *H. pylori* infection, suggesting that this enzyme is induced by inflammation and may have an important role in the natural history of human gastritis.

Keywords: Gastritis, gastric inflammation, *Helicobacter pylori* virulent strains

***Experimental Biology and Medicine* 2018; 243: 1161–1164. DOI: 10.1177/1535370218819423**

Introduction

Transglutaminases (R-glutaminyl-peptide: amine γ-glutamyl transferase: EC2.3.2.13) are a family of nine isoenzymes which catalyze transamidations, or crosslinking reactions, in which an amide reacts with a primary amino group of lysine residues to form new amide bonds.[1] Tissue-transglutaminase (t-TG) is unique among TG enzymes because of its additional role in several physiological processes. It was first described by Sarkar *et al.*, and has been extensively studied since its discovery.[2,3] It is ubiquitously distributed in mammals and is detectable both inside the cell and extracellularly in various types of tissues.[4] Its activity depends on its cellular location and conformation.[3] In normal conditions, t-TG in the cytosol and extracellular matrix is predominantly in a closed conformation, maintained by guanine nucleotide and/or integrin

bindings. Physical or chemical injury opens the conformation and activates the enzyme.[5] The enzyme is calcium-dependent and covalently cross-links a variety of proteins in the extracellular matrix, increasing fibrosis in order to favor wound healing by increasing resistance to chemical, enzymatic, and physical disruption.[6]

Infection with *Helicobacter pylori* is common worldwide. The presence of polymorphonuclear cells in the gastric mucosa among normal mononuclear cell infiltrate is a hallmark of *H. pylori* infection. The severity of gastritis may vary in different sites of the stomach, and is typically most severe in the non-acid secreting portions of the stomach such as the antrum and the cardia.[7] Fibrosis and architectural distortion may be part of the morphological changes seen in patients with long lasting *H. pylori* gastritis. Differences between *H. pylori* strains have been noted, and are thought to be related to the presence of virulence factors. Strains encoding the cytotoxin-associated gene (cag) pathogenicity island, including cytotoxin-associated gene A (cagA), co-express vacuolating cytotoxin (vacA).[8] *H. pylori* strains that produce cagA and vacA cause intense cell injury.[9,10] The presence of polymorphisms in the vacA gene makes bacteria isolates different from each other.[11] The three variable regions identified are: *s*-region (signal sequence region); *m*-region (mid-region); and the *i*-region (intermediate-region). There are two allelic variants in the *s*-region named *s1* and *s2,* and two variants in the *m*-region called *m1* and *m2*.[11] Different combinations of *s* and *m* genotypes are associated with the severity of chronic inflammation. For example, *H. pylori* vacA *s1/m1* and *s1/m2* strains are more virulent compared to the other genotypes and are associated with an increased risk of gastric cancer.[11]

The aim of this study was to measure gastric t-TG activity in patients with dyspepsia according to *H. pylori* infection and cagA, vacA-*s*, and vacA-*m* status.

Materials and methods

Study population

Patients referred to the Digestive Endoscopy Service, Department of Internal Medicine, University of Sassari, Italy, for dyspeptic symptoms and undergoing esophago-gastro-duodenoscopy (EGD) were asked to participate in the study.

A gastroenterologist collected the clinical history from each patient when they were due to undergo their EGD. All relevant data including demographic information, digestive symptoms, treatments, co morbidities, and any previous treatment for *H. pylori* were recorded. Patients who underwent EGD for reasons other than dyspepsia (e.g. cirrhosis follow-up, suspected celiac disease, alarm symptoms), those with a prior history of upper gastrointestinal surgery, severe heart or kidney disease, currently pregnant or lactating, active malignancy, and those taking medications were not included in this study.

Histology

Biopsy specimens were obtained in each patient from the antrum (2), the angulus (1), and the corpus (1) and stored in separate vials for subsequent histology analysis. Biopsy specimens were stained with hematoxylin–eosin and Giemsa stains, and morphology was assessed by an expert GI-pathologist as previously described.[12] Three additional biopsies were collected from the antrum: one was used for the rapid urease test (RUT), one for microbial culture and one for t-TG determination.

H. pylori infection was defined by the detection of bacteria on gastric specimens and a positive RUT. Positive and negative *H. pylori* status was confirmed by a 13C urea breath test.

Ethical considerations

Institutional Review Board approval was obtained from the *Comitato di Bioetica, Azienda Ospedaliero-Universitaria di Sassari* (Prot N° 2099/CE).

Bacterial culture

Selective brain–heart infusion agar with 7% defibrinated horse blood agar plates were inoculated with 100 μL of Brucella broth containing the homogenized gastric specimens as previously reported.[13]

Molecular typing

To detect virulence factors in *H. pylori* strains, genomic DNA was extracted, using the QIAmp tissue Kit (QIAGEN, Chatsworth, CA) according to the manufacturer's instructions and amplified using the polymerase chain reaction. Specific oligoprimers for each gene sequence were used for PCR amplification of the cagA, vacA-*s*, and vacA-*m* regions.[13]

Determination of t-TG activity

The enzyme activity in antral mucosa homogenates was assessed using a modified radiometric method.[14,15] Briefly, specimens of gastric mucosa were homogenized in 20 mmol/L Tris-HCl at 4°C mixed with 0.3 mmol/L putrescine, 1 μCi ^{3}H–putrescine, 50 mmol/L dithiothreitol, 10 mmol/L $CaCl_2$ and 4% dimethyl-casein, and radioactivity detected using the standard filter paper assay. The activity was expressed as putrescine pmol/mg of proteins/minute. Each specimen was assessed by an operator blinded to the *H. pylori* status.

Data analysis

The results were expressed as mean ± standard deviation for scalar variables, or as frequencies for categorical variables. The non-parametric Mann–Whitney U test was used for comparing the t-TG activity between patients with *H. pylori* positive and *H. pylori* negative gastritis. Statistical analysis was conducted using SPSS Statistical Package (version 16.0, Chicago, IL) and the results were considered significant when P values were less than 0.05.

Results

A total of 46 patients with dyspepsia (mean age 47.8 ± 15.2 years) consented to participate in the study. All patients

were from Northern Sardinia, with an overall frequency of *H. pylori* infection of 36.9% (17 out of 46), 30 patients were female (Table 1). *H. pylori* infection was associated with chronic active gastritis in 17 patients and metaplasia and/or atrophy was present in addition to gastritis in 11 patients. Of note, two of the patients with dyspepsia had normal gastric mucosa during the histological examination of the specimens (Table 1).

The Mann–Whitney U test showed that t-TG activity in *H. pylori*-positive patients was significantly greater compared with *H. pylori*-negative patients (6437 ± 3691 vs. 3773 ± 1530 pmol/mg; $P = 0.001$). Tissue-TG in the *H. pylori*-negative group was slightly higher than the group with normal mucosa (3773 ± 1530 vs. 3115 ± 655 pmol/mg; Table 1). However, the small number of patients ($n = 2$) without gastritis did not allow additional analyses.

H. pylori strains were found to be positive for cagA in 13 (72%) cases. The activity of t-TG was higher in patients harboring cagA positive strains vs. cagA negative strains (7358 ± 4318 vs. 4895 ± 1062 pmol/mg; $P = 0.237$). Five *H. pylori* strains were *s1/m1*-, six were *s1/m2*-, and five were *s2/m2*-vacA. Bacteria strains with *s2/m1*-vacA were not detected. *H. pylori s1/m1*-vacA strains displayed the highest t-TG enzyme activity (7429 pmol/mg) compared to *s1/m2*-vacA (5045 pmol/mg) and *s2/m2*-vacA (4489 pmol/mg) *H. pylori* strains, respectively, although the differences were not statistically significant. There were no differences between histology, endoscopy features and t-TG activity.

Discussion

Our results show that t-TG activity was significantly higher in homogenates from antral specimens from patients with chronic active gastritis associated with *H. pylori* infection compared to *H. pylori* negative chronic gastritis and normal antral mucosa. Although the presence of t-TG has previously been described in human and animal models of the intestine,[16,17] the presence of t-TG has never to the best of our knowledge been described in the stomach in relation to *H. pylori* infection. Previous indirect results came from a study by Borghini *et al*.[18] Among 18 patients with celiac disease, the authors did not find significant differences between anti-t-TG levels and anti-endomysium in the supernatant cultures of gastric biopsies separated according to *H. pylori* status. The apparent contrast with our results may be explained by the different methods used in the studies: the enzyme was detected by using antibodies in the study by Borghini *et al*., while t-TG activity was measured directly in our study. Tissue-TG is expressed at sites of inflammation,[6] and can act as a modulator of inflammation, exerting both pro- and anti-inflammatory effects.[19] *H. pylori*, once acquired, is able to cross the gastric mucus layer and proliferate. A proportion of bacteria adheres to the mucosal cells and others penetrate the space between cells. The direct contact between *H. pylori* and the gastric cells stimulates the synthesis and release of chemokines, including IL 8, resulting in a marked infiltration of neutrophils.[20] In addition to neutrophils, it has been demonstrated that monocytes are also important as a source of proinflammatory mediators such as IL-1 and TNF-α, and for generating reactive oxygen species and nitric oxide in response to *H. pylori* infection.[21,22] Therefore, it seems reasonable to find increased levels of t-TG in the antral mucosa of patients with an active-chronic gastritis positive for *H. pylori*. For example, Wang *et al*., observed that hypertonic NaCl-induced gastric mucosal damage was associated with a significant increase in t-TG activity in animal models and concluded that increased transglutaminase activity was involved in the mechanism of mucosal healing.[23] In their elegant study, Haroon *et al*. reported that t-TG was activated and directly involved in tissue repair.[24] Our results obtained *in vivo* corroborate the findings observed in animal models. In the three groups of patients studied in this experiments: (i) *H. pylori* positive active-chronic gastritis, (ii) *H. pylori* negative chronic gastritis, and (iii) normal gastric mucosa, there was a trend towards decreasing concentrations of t-TG in the antral specimens with decreasing gastritis and *H. pylori* infection. Gastric epithelial degeneration is not specific to *H. pylori* infection and may be observed in all types of gastritis. In accordance with this, we found higher levels of t-TG in *H. pylori* negative gastritis when compared to normal mucosa.

It has been extensively reported that *H. pylori* strains producing cagA and vacA*s1/m1* and *s1/m2* cause more intense tissue inflammation and increased cytokine production, resulting in greater epithelial damage.[9,10] This notion is confirmed by our results, having found that t-TG activity was higher in positive cagA gastritis compared to negative cagA gastritis. We can suppose that the lack of difference in t-TG activity between virulent and non-virulent *H. pylori* strains was blurred by the vigorous inflammation induced by infection with any strain of *H. pylori*.

In conclusion, for the first time we show that t-TG activity is significantly higher in chronic active gastritis associated with *H. pylori* infection and with gastritis in general, suggesting that t-TG activity is induced by inflammation

Table 1. Levels of tissue transglutaminase (t-TG) activity according to *H. pylori* status and gastritis in dyspeptic patients.

Variable	*H. pylori* positive gastritis	*H. pylori* negative gastritis	Normal gastric mucosa
Patient number	17	27	2
Sex			
Male/Female	9/8	7/20	0/2
Age	52.9 ± 13.2	45.7 ± 15.8	31.5 ± 10.6
Presence of ulcer/erosion at the endoscopy	1	2	0
Presence of metaplasia and/or atrophy at histology	11	7	0
TG (homogenate of gastric mucosa)	705 ± 510	450 ± 220	200 ± 231
t-TG (pmol of ^{3}H-putrescine/mg of protein)	6437 ± 3691[a]	3773 ± 1530[b]	3115 ± 655

[a]Tissue-TG activity in *H. pylori*-positive patients differed significantly from [b]*H. pylori*-negative patients ($P = 0.001$).

and may have a role in the natural history of human gastritis. The importance of this observation in the pathogenesis of *H. pylori* positive gastritis requires further investigation.

Author contributions: All authors participated in study design, interpretation of results, analysis of the data, and review of the manuscript; MPD, AE, GMP, and AM conducted the experiments; MPD and GMP wrote the manuscript.

DECLARATION OF CONFLICTING INTERESTS

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING

The authors received no financial support for the research, authorship, and/or publication of this article.

ORCID iD

Giovanni Mario Pes http://orcid.org/0000-0003-3265-2823

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(Received October 7, 2018, Accepted November 25, 2018)