Impairment of Salivary Mucin Production Resulting in Declined Salivary Viscosity During Naproxen Administration as a Potential Link to Upper Alimentary Tract Mucosal Injury

Cesar J. Garcia, MD\textsuperscript{1}, Juan Castro-Combs, MD\textsuperscript{1}, Ajoy Dias, MD\textsuperscript{1}, Rodrigo Alfaro, MD\textsuperscript{1}, Javier Vasallo, MD\textsuperscript{1}, Marek Majewski, MD\textsuperscript{1}, Tom Jaworski, MD\textsuperscript{2}, Grzegorz Wallner, MD\textsuperscript{2} and Jerzy Sarosiek, MD, PhD, AGAF, FACG\textsuperscript{1}

OBJECTIVES: Nonsteroidal anti-inflammatory drugs (NSAIDs) contribute to the esophageal mucosal injury through its direct topical impact on the luminal aspect of the surface epithelium. Its indirect, systemic impact, however, on salivary component of the esophageal pre-epithelial barrier remains to be explored. Therefore, salivary mucin secretion and viscosity at baseline and during naproxen-placebo, as well as naproxen-rabeprazole, administration were investigated.

METHODS: Twenty-one asymptomatic volunteers were included in this double-blind, placebo-controlled, crossover designed study. Salivary samples were obtained in basal and pentagastrin-stimulated conditions (6 mg/kg s.c.) mimicking the food-stimulated conditions. Patients received 7 days of naproxen-placebo or naproxen-rabeprazole with a 2-week washout period in between. Salivary mucin content and viscosity were measured before and after treatment using periodic acid/Schiff’s methodology and Cone/Plate Digital Viscometer, respectively.

RESULTS: The rate of salivary mucin secretion in basal condition declined by 32% during administration of naproxen-placebo (11.3 ± 1.7 vs. 16.8 ± 3.3 mg/h). Salivary mucin secretion in pentagastrin-stimulated condition declined significantly (by 34%) during the administration of naproxen-placebo (13.6 ± 1.5 vs. 20.7 ± 3.0 mg/h; \( P < 0.05 \)). Viscosity significantly decreased after naproxen-placebo administration in basal (by 60%) and stimulated conditions (by 56%) \( (P < 0.001) \). Coadministration of rabeprazole at least partly restored the naproxen-induced decline of salivary mucin in basal condition (by 8%), and pentagastrin-stimulated conditions (by 30%).

CONCLUSIONS: A significant decline of salivary mucin and viscosity during administration of naproxen may at least partly explain a propensity of patients on chronic therapy with NSAIDs to the development of esophageal mucosal injury and complications. In addition the trend to restorative capacity of rabeprazole on the quantitative impairment of salivary mucin during administration of naproxen may potentially translate into its tangible clinical benefit but it requires further investigation.

Clinical and Translational Gastroenterology (2013) 4, e40; doi:10.1038/ctg.2013.8; published online 25 July 2013

Subject Category: Stomach

INTRODUCTION

It is well known that the treatment with nonsteroidal anti-inflammatory drugs (NSAIDs) represents a challenge that requires individualized patient evaluation of risks vs. clinical benefits. Common treatment risks such as history of peptic ulcer or bleeding, especially in patients with advanced age, multiple comorbidities, and comedication with aspirin or warfarin require serious attention.\textsuperscript{1–5} Chronic NSAIDs administration may result in the development of esophageal mucosal injury through its direct topical impact on the luminal aspect of the surface epithelium or by the effects exerted systemically.

The integrity of the upper alimentary tract mucosa depends upon the equilibrium between aggressive factors and protective mechanisms.\textsuperscript{6–9} Hydrogen ions (H\textsuperscript{+}) represent the major aggressive factor in mucosal injury and its pharmacological control poses an impressive challenge for both general practitioners and gastroenterologists.\textsuperscript{8,9} Secretion of gastric acid into the lumen is followed by back-diffusion of hydrogen ion toward the mucosa, a phenomenon that remains strictly concentration dependent.\textsuperscript{7,11–12}

This back-diffusing hydrogen ion is counterbalanced by continuous renewal of the mucus–buffer layer covering the surface epithelium of the upper alimentary tract mucosa that is gradually eroded by acid–pepsin due to proteolytic activity on its luminal aspect. The protective quality of this mucus–buffer layer is greatly affected by NSAIDs and is at least partially mediated by inhibition of prostaglandin generation mediated by cyclooxygenase-1 enzyme.\textsuperscript{6,7,13} The quality and quantity of this layer are strongly influenced by secretions from salivary gland. Salivary protective qualities, defined by its viscosity, gel-forming quality and its capacity to lubricate during swallowing, are well known for their protective potential within the oral cavity, as well as at the esophageal and gastric mucosal compartments.\textsuperscript{14}

\textsuperscript{1}Department of Internal Medicine, Mol. Med. Res. Lab., Texas Tech University Health Sciences Center, El Paso, Texas, USA and \textsuperscript{2}Medical University of Lublin, Lublin, Poland

Correspondence: Jerzy Sarosiek, MD, PhD, AGAF, FACG, Department of Internal Medicine, Mol. Med. Res. Lab., Texas Tech University Health Sciences Center, 4800 Alberta Avenue, El Paso, Texas 79905, USA. E-mail: jerzy.sarosiek@ttuhsc.edu

Received 15 September 2012; accepted 9 May 2013
Rabeprazole is well known for its proton pump (H⁺/K⁺ ATPase) inhibitor (PPI) activity that profoundly diminishes gastric acid secretion, lowering the luminal concentration of hydrogen ions. It has been shown that rabeprazole is the only PPI among tested (omeprazole, lansoprazole) that augments gastric mucus and mucin secretion in experimental animals, finding that have recently been confirmed in humans subjects. Furthermore, it has been recently demonstrated that the administration of naproxen produces a significant decline in gastric mucin production, the major component of the protective mucous–buffer layer. No wonder that the coadministration of naproxen produces a significant decline in gastric mucin production, the major component of the protective mucous–buffer layer. No wonder that the coadministration of naproxen produces a significant decline in gastric mucus and mucin secretion in experimental animals, finding that have recently been confirmed in humans subjects.1

As the content of mucin is the major factor determining the viscosity of the alimentary tract secretions, parallel measurements of viscosity and mucin could provide a valuable assessment of the protective quality of saliva during naproxen administration. We have investigated, salivary mucin secretion and viscosity in asymptomatic volunteers being treated with naproxen, and the potential restorative impact of rabeprazole coadministration in salivary mucin production in a double-blind, placebo-controlled, crossover designed study protocol.

METHODS

Subjects. This study was approved by the Human Subject Committee. All investigated subjects provided informed consent to the experimental procedure. Twenty-one asymptomatic volunteers (11 females and 10 males; mean age of 34 years; 19–58 range) were enrolled in this study protocol, which was designed as a double-blind, placebo-controlled, crossover study. All volunteers were randomly assigned to 1 week of naproxen (500 mg b.i.d.) combined with rabeprazole (20 mg q.d.) or placebo (20 mg q.d.) with a 2-week washout period in between.

Sample collection and analysis. Samples of saliva were collected at baseline (before therapy) and at the end of both administered treatments. On the 7th day of assigned treatment, the samples were collected after an overnight fast. The last treatment dose was administered 1.5 h before the saliva sample collection procedure. Saliva was collected during 1 h in basal conditions and 1 h after administration of pentagastrin (6 μg/kg s.c.), mimicking the natural food-stimulated conditions scenario.

The content of salivary mucin was measured with periodic acid/Schiff’s methodology. The standard curve was performed utilizing purified (ultracentrifugation at 280,000 g for 48 h in CsCl) human salivary mucin. Viscosity (mPa s) was recorded using Cone/Plate Digital Viscometer (Brookfield, Stoughton, MA) with eight consecutive share rates between 0.3 and 60 r.p.m. range, representing minimal and maximal share stress taking place during the physiology of chewing and swallowing of the bolus of solid food.

Table 1 The rate of mucin secretion in saliva collected in basal conditions and after administration of pentagastrin, mimicking the food-stimulated conditions, before (baseline) and after administration of naproxen/placebo and/or naproxen/rabeprazole in asymptomatic volunteers (n = 21)

| Parameter          | Basal     | %     | Pentagastrin-stimulated conditions | %     | P vs. Bline |
|--------------------|-----------|-------|------------------------------------|-------|-------------|
| Bline              | 16.8 ± 3.3| 100%  | 20.7 ± 3.0                         | 100%  |             |
| N/P                | 11.3 ± 1.7| –32%  | 13.2 ± 1.5                         | –34%  | <0.05       |
| N/R                | 12.3 ± 2.0| –8%   | 17.2 ± 2.6                         | –30%  | NS          |

Abbreviations: Bline, baseline; N/P, naproxen/placebo; N/R, naproxen/rabeprazole; NS, not significant.
shear rate. The average decline of salivary viscosity recorded within eight consecutive shear rates during naproxen administration was 59% ($P<0.001$) (Table 2).

The salivary viscosity after stimulation with pentagastrin was 130.8 ± 26.4 mPa s at the lowest and 3.3 ± 0.3 mPa s at the highest shear rate. The viscosity value of salivary secretion, however, after administration of naproxen, declined by 60% (70.8 ± 18.0 mPa s, $P<0.01$) at the lowest shear rate and declined only by 16% (2.8 ± 0.2 mPa s) at the highest shear rate. The average decline of salivary viscosity recorded within eight consecutive shear rates during naproxen administration was 46% ($P<0.01$). As administration of rabeprazole plus naproxen did not result in any changes in viscosity, to make our tables simpler for potential readers we omitted them from the Tables 2 and 3.

**DISCUSSION**

Acetylsalicylic acid was the first NSAID synthesized in 1897. A long time has passed and NSAIDs have become one of the most common prescription drugs in the world.18,19 Despite the anti-inflammatory and analgesic properties of these agents, they are not innocuous and can produce serious adverse effects, including upper gastrointestinal tract ulcers and vascular complications. They produce acute damage of the gastric mucosa in up to 100% of the patients after short-term use. Fortunately, during chronic administration, due to an adaptive phenomenon within the alimentary tract mucosa, the incidence of peptic ulcer disease is only 10–15%.20

This injury is produced by two mechanisms. One is by topical injury, produced by the conversion to their ionized form and producing direct damage to the mucosa.21 The second mechanism of injury results from systemic effect, associated with inhibition of cyclooxygenase-1 and diminished release of prostaglandins, which have an important role in the secretion of mucus and bicarbonate, epithelial proliferation and increased mucosal blood flow, thus, preventing broad spectrum of NSAID-related mucosal injury and complications.22 Naproxen is non selective NSAID that produces a significant inhibitory effect on both cyclooxygenase isoenzymes.1,23

Gastroprotection needs to be contemplated in every patient with high risk of NSAID-related ulcers.23,24 There are several agents proposed to achieve this goal: misoprostol, a prostaglandin analog, has proven to be effective in preventing NSAID-related gastric and duodenal ulcers, as well as reducing the risk of complications.25–28 However, its dosing (QID) and common side effects makes this drug less appealing to our patients. Ranitidine and famotidine have a modest impact on preventing duodenal ulcers but are not proven to be beneficial in successful prevention of gastric ulcers.28–31

PPIs have shown to be superior to H2 receptors antagonists and prostaglandin analogs in protecting the gastrointestinal tract from NSAID damage.24,32 PPIs, such as rabeprazole, lansoprazole, omeprazole and pantoprazole, reduce gastric acid secretion by inhibiting the proton pumps in stimulated gastric parietal cells, producing an increase of the pH of the stomach.33 Results from five randomized clinical trials demonstrated that chronic NSAID users treated with PPIs had a 14.5% rate of duodenal and gastric ulcers by endoscopic surveillance compared with 35% in the placebo group.28 In addition to their higher success in preventing NSAID-related mucosal damage, they promote healing of NSAID-induced damage.34 Furthermore, PPIs have an excellent safety profile,35 making PPIs the drug of choice for NSAID-induced ulcers.36,37 Rabeprazole has in its molecule hydrophobic component that interacts with hydrophobic...
structures of mucin-secreting cells, resulting in subsequent release mucin granules stored within mucin-secreting cells.1

Mucin has been proposed as the main protective agent against acid–pepsin.38 Secretion of mucin is mediated by prostaglandine E2. It serves as a protective coat to the gastric mucosa, maintaining a stable pH and minimizing direct enzymatic attack by pepsin.37 NSAIDs by inhibiting the cyclooxygenase-1 and prostaglandine E2 production interfere with the mucin secretion, making the gastric mucosa vulnerable to damage by gastric acid and pepsin.23

Recently, it has been demonstrated that coadministration of rabeprazole with naproxen significantly restores gastric mucin impairment induced by naproxen.1 The potential restorative impact of rabeprazole administration on naproxen-induced salivary mucin and mucin-related viscosity impairment remained to be explored.

We demonstrated for the first time that the administration of naproxen resulted in a pronounced and significant decline in salivary viscosity in both basal conditions and after pentagastrin stimulation (by 59.9% and 55.9%, respectively) at the lowest shear rate (0.3 r.p.m.), and it also significantly decreased the basal (by 38%) and after pentagastrin stimulation (by 16%) at the highest shear rate (60 r.p.m.). The average of significant decline of salivary viscosity recorded within eight consecutive shear rates was 59.5% at baseline and 45.7% after pentagastrin. We also demonstrated salivary mucin profound production impairment in basal (by 32%) and significant decline in pentagastrin-stimulated (by 34%) conditions, mimicking the natural food-stimulated conditions scenario. Eighteen subjects (of 21 tested) responded with a decline in salivary mucin output in basal or stimulated conditions, and 10 subjects exhibited diminished mucin secretion in both basal and stimulated conditions simultaneously. One may hypothesize that some subjects with a decline in salivary mucin output in both basal and stimulated conditions, simultaneously, are potential candidates for the development of alimentary tract complications. However, this hypothesis would require a prospective long-term clinical study. If it is confirmed, this could help to predict which patients on chronic NSAIDs therapy will require PPIs by running a simple salivary mucin test in freshly collected saliva.

We also demonstrated that coadministration of rabeprazole with naproxen has a restorative impact on the salivary mucin production impairment revealed during administration of naproxen with placebo, similar to that reported in gastric mucin secretion study.1 The salivary mucin output during administration of naproxen/rabeprazole combination increased by 30% in pentagastrin-stimulated conditions from the naproxen/placebo combination, although did not reach statistical significance. This restorative impact of rabeprazole on salivary mucin production impairment induced by administration of naproxen could have some beneficial impact on the protective quality of the mucus–buffer layer covering the surface of the epithelium of the upper alimentary tract, which provides mucosal defense against luminal mechanical and chemical injury. Salivary gland secretion has a significance role in the maintenance of the integrity of the gastric mucosa in experimental animals39 and may justify further studies in humans employing the randomized, placebo-controlled study protocol during chronic therapy with NSAIDs and rabeprazole coadministration confirmed endoscopically.

Salivary mucin, as well as gastric and esophageal mucins, is released by the mucous cells. This process is stimulated by different signal pathways including cholinergic, histaminergic, and peptidergic among others. Mucin is deposited in the surface of the mucosa and generates thicker mucus that buffers the gastric acid in order to maintain a stable pH. The capacity of the mucus to act as a buffer depends directly on its thickness.7,40 High content of mucin within the alimentary tract secretion determined the highly viscous and adhesives properties. This results in accumulation of secretions on the surface epithelium setting the stage for creation of the mucous–buffer layer.7

This mucous–buffer layer owing to its accumulation of buffers continuously secreted by epithelium generates a pH gradient from an acid value on its luminal aspect of the gastric mucosa and neutral pH at surface epithelium cell membrane. This is of a great protective value, especially, in the upper gastrointestinal tract, where gastric acid is a continuous challenging to the surface of the epithelium.41

Drug-induced esophageal injury has been reported with many different medications. Tetracyclines, bisphosphonates, NSAIDS, potassium chloride and quinine are among the most common medications implicated in esophageal complications. Complications may vary from inflammation, ulceration, stricture, malnutrition to more serious conditions such as hemorrhage, perforation, and death. Most patients with esophageal complications do not have identifiable risk factors making them unpredictable. The most common mechanism of injury shared by most medications is the prolonged contact between the pill and the esophageal mucosa. A normal salivary composition and output may increase lubrication during swallowing, and this reduces the time of exposure of esophageal mucosa to these agents, decreasing the risk of serious complications.42 It is noteworthy that the removal of salivary gland secretion results in significant decline of the functional integrity of the esophageal mucosa in an experimental animal model.43 The impact, however, of NSAIDs therapy on esophageal mucin secretion in humans remains to be confirmed.

The decline of salivary mucin content and viscosity could potentially be an objective screening test to determine which patients are at high risk of developing NSAIDs associated ulcer and/or esophageal complications. Diminished viscosity does not affect the practical aspect of testing for the content of mucin in corresponding samples, but hampers protective quality of the alimentary tract secretions. This testing, therefore, could potentially also allow to determine the degree of mucosal damage prior the implementation of therapy and an important tool to evaluate the response to the therapy with PPIs, particularly with rabeprazole. This requires, however, further investigations in humans.

In conclusion, a significant decline of salivary mucin and viscosity during administration of naproxen may at least partly explain a propensity of patients on chronic therapy with NSAIDs to the development of esophageal mucosal injury and complications. In addition the trend to restorative capacity of rabeprazole on the quantitative impairment of salivary mucin during administration of naproxen may potentially translate
into its tangible clinical benefit but it requires further investigation.

CONFLICT OF INTEREST
Guarantor of the article: Jerzy Sarosiek, MD, PhD, AGAF, FACC.
Specific author contributions: Planning and/or conducting the study: Jerzy Sarosiek; collecting and/or interpreting data: Ces\a r J. Garcia, Ajoy Dias, Rodrigo Alfaro, Marek Majewski, Tom Jaworski, and Grzegorz Wallner; drafting the manuscript: Cesar J. Garcia, Ajoy Dias, Rodrigo Alfaro, Juan Castro-Combs, and Javier Vasallo.
Financial support: None.
Potential competing interests: None.

Acknowledgements. We thank Dr Richard McCallum for his encouragement.

Study Highlights

WHAT IS CURRENT KNOWLEDGE
- The pathomechanism of non-steroidal anti-inflammatory drugs (NSAIDs)-induced injury to the alimentary tract mucosa is multifactorial.
- It involves both systemic factors such as inhibition of cyclo-oxygenase-2 (COX-2) activities resulting in decline of prostaglandins as well as local mucosal drop in prostaglandin cytoprotection within the mucosal surface epithelium induced by blocking COX-1 enzyme.
- This is accompanied by the hampered rate of mucin and mucus secretion compromising the protective quality of the mucus-buffer layer covering the alimentary tract mucosa and serving as a vanguard of mucosal protection.

WHAT IS NEW HERE
- We are demonstrating for the first time in humans that administration of naproxen to asymptomatic volunteers resulted in significant decline of salivary mucin secretion accompanied by significant decline of salivary viscosity.
- Since salivary secretion is the major protective component within the esophageal pre-epithelial barrier, any decline of its protective quality may facilitate the development of esophageal mucosal injury in chronic NSAID users.
37. Singh G, Triadafilopoulos G. Appropriate choice of proton pump inhibitor therapy in the prevention and management of NSAID-related gastrointestinal damage. Int J Clin Pract 2005; 59: 1210–1217.

38. Niv Y. H. pylori/NSAID—Negative peptic ulcer—The mucin theory. Med Hypotheses 2010; 75: 433–435.

39. Sarosiek J, Bilski J, Murty VL et al. Role of salivary epidermal growth factor in the maintenance of physicochemical characteristics of oral and gastric mucosal mucus coat. Biochem Biophys Res Commun 1988; 16: 1421–1427.

40. Phillipson M, Johansson ME, Henikanss J et al. The gastric mucus layers: constituents and regulation of accumulation. Am J Physiol Gastrointest Liver Physiol 2008; 295: 806–812.

41. Allen A, Flemstrom G, Garner A et al. Gastrroduodenal mucosal protection. Physiol Rev 1993; 73: 823–857.

42. O’Neill Jessica, Remington Tami. Drug-induced esophageal injuries and dysphagia. Ann Pharmaco Ther 2003; 37: 1675–1684.

43. Sarosiek J, Feng T, McCallum RW. The interrelationship between salivary epidermal growth factor and the functional integrity of the esophageal mucosal barrier in the rat. Am J Med Sci 1991; 302: 359–363.

Clinical and Translational Gastroenterology is an open-access journal published by Nature Publishing Group. This work is licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License. To view a copy of this license, visit http://creativecommons.org/licenses/by-nc-sa/3.0/