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

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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 $\pm$ 1.7 vs. 16.8 $\pm$ 3.3 mg/h). Salivary mucin secretion in pentagastrin-stimulated condition declined significantly (by 34%) during the administration of naproxen-placebo (13.6 $\pm$ 1.5 vs. 20.7 $\pm$ 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.

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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.[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.[6–9] Hydrogen ions (H^+) represent the major aggressive factor in mucosal injury and its pharmacological control poses an impressive challenge for both general practitioners and gastroenterologists.[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.[7,10–12]

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.[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.[14]

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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.[15] It has been shown that rabeprazole is the only PPI among tested (omeprazole, lansoprazole) that augments gastric mucus and mucin secretion in experimental animals,[16] finding that have recently been confirmed in humans subjects.[17] 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 rabeprazole and naproxen significantly restores the profound impairment of gastric mucin secretion induced by naproxen.[1] However, the impact of naproxen and rabeprazole treatment on salivary mucin secretion remains to be determined.

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.

Data processing and statistical analysis. Statistical analysis was performed using ∑-Stat software (SyStat, San Jose, CA). Data are presented in basal conditions and after stimulation with pentagastrin. All results are expressed as mean ± s.e. for data with a normal distribution after subsequent statistical analysis using parametric tests for data distributed normally, as recommended by professional ∑-Stat software.

RESULTS

The output of salivary mucin during administration of naproxen/placebo combination declined by 32% in basal (11.3 ± 1.7 vs. 16.8 ± 3.3 mg/h) and by 34% in pentagastrin-stimulated (13.6 ± 1.5 vs. 20.7 ± 3.0 mg/h; $P<0.05$) conditions (Table 1).

Out of 21 investigated subjects, 18 responded by the decline in salivary mucin output in basal or stimulated conditions (decrease in mucin output between 20 and 87%) and 10 subjects exhibited diminished mucin secretion in both basal and stimulated conditions.

The salivary mucin output during administration of naproxen/rabeprazole increased in basal condition by 8% (12.3 ± 2.0 vs. 11.3 ± 1.7 mg/h) and in pentagastrin-stimulated condition by 30% (17.2 ± 2.6 vs. 13.6 ± 1.5 mg/h) from the corresponding values revealed during naproxen/placebo administration (Table 1).

Administration of naproxen/rabeprazole resulted in an increase in salivary mucin output by >50% vs. the corresponding naproxen/placebo valued in seven subjects.

The viscosity value of salivary secretion in basal condition was 72.7 ± 9.7 mPa s at the lowest (0.3 r.p.m.) and 3.6 ± 0.3 mPa s at the highest shear rate (60 r.p.m.) of the digital viscometer. The viscosity value of salivary secretion, however, after administration of naproxen, declined by 59.9% (29.1 ± 3.0 mPa s, $P<0.001$) at the lowest shear rate and declined by 38% (2.2 ± 0.1 mPa s, $P<0.001$) at the highest

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	Mucin output (mg/h)				$P<$ vs. Bline
	Basal	%	Pentagastrin-stimulated conditions	%	
Bline	16.8 ± 3.3	100%	20.7 ± 3.0	100%	
N/P	11.3 ± 1.7	− 32 vs. Bline	13.2 ± 1.5	− 34 vs. Bline	<0.05
N/R	12.3 ± 2.0	+ 8 vs. N/P	17.2 ± 2.6	+ 30 vs. N/P	NS

Abbreviations: Bline, baseline; N/P, naproxen/placebo; N/R, naproxen/rabeprazole; NS, not significant.

Table 2 Salivary secretion viscosity before (baseline) and during naproxen/placebo administration in basal conditions

	Basal saliva							
Baseline (share rates, r.p.m.)	0.3	0.6	1.5	3	6	12	30	60
Mean (mPa s)	72.7	41.5	23.5	14.7	10.0	7.3	5.0	3.6
± s.e.	9.7	6.0	4.0	2.3	1.5	1.0	0.6	0.3
N&P	0.3	0.6	1.5	3	6	12	30	60
Mean (mPa s)	29.1	16.2	8.9	6.3	4.7	3.8	2.8	2.2
± s.e.	3.0	1.9	1.2	0.9	0.6	0.4	0.3	0.1
$P<$ vs. baseline	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Abbreviation: N&P, naproxen and placebo.

Table 3 Salivary secretion viscosity before (baseline) and during naproxen/placebo administration in stimulated conditions (administration pentagastrin, mimicking the food-stimulated conditions)

	Stimulated saliva							
Baseline (share rates, r.p.m.)	0.3	0.6	1.5	3	6	12	30	60
Mean (mPa s)	130.8	71.3	38.4	22.6	14.1	8.8	4.6	3.3
± s.e.	6.5	14.1	7.9	4.2	2.2	1.2	0.4	0.3
N&P	0.3	0.6	1.5	3	6	12	30	60
Mean (mPa s)	70.8	39.2	19.3	12.7	9.0	5.2	3.6	2.8
± s.e.	18.0	10.0	4.4	2.7	1.9	0.5	0.3	0.2
$P<$ vs. baseline	<0.01	<0.01	<0.001	<0.01	<0.05	<0.001	<0.01	<0.05

Abbreviation: N&P, naproxen and placebo.

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 results 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.[1,2]

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, lanzoprazole, 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 animals[39] 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, FACG.
Specific author contributions: Planning and/or conducting the study: Jerzy Sarosiek; collecting and/or interpreting data: Cesar 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.
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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 cyclooxygenase-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.

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