Increased gastric juice epidermal growth factor after non-steroidal anti-inflammatory drug ingestion

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Abstract

Epidermal growth factor (EGF), present in saliva and gastric juice, is a potent mitogen and an important element of mucosal defence. Changes in salivary and gastric juice epidermal growth factor in response to non-steroidal anti-inflammatory drug (NSAIDs) ingestion were measured to assess the role of EGF in gastric mucosal adaptation to NSAIDs. Patients with arthritis underwent endoscopy with collection of saliva and gastric juice for EGF measurement, before and two weeks after continuous NSAID ingestion. During this period patients also received either the prostaglandin analogue misoprostol or placebo in addition to their NSAID. In the misoprostol group (n=5) there was no observed mucosal damage and no change in either salivary or gastric juice EGF. In the placebo group (n=10) three patients developed erosions. Salivary EGF did not change (mean (SEM) 3.02 (0.54) ng/ml v 2.80 (0.41) ng/ml) but gastric juice EGF increased from 0.42 (0.12) ng/ml to 0.69 (0.14) ng/ml ($p < 0.05$). This increased EGF could contribute to the increased cellular proliferation observed during NSAID ingestion and may represent an important mechanism underlying gastric mucosal adaptation.

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It is now well known that non-steroidal anti-inflammatory drugs (NSAIDs) damage the gastroduodenal mucosa leading to mucosal erosion and ulceration.¹⁻³ They are also associated with an increased incidence of ulcer-related complications such as perforation.^{4,5} Endoscopic studies have shown gastric mucosal damage during the first four weeks of NSAID administration. These studies have also shown, however, that mucosal lesions may improve, and even heal completely, despite continued NSAID ingestion.⁶⁻⁸ The mechanisms involved in this 'adaptation' of the gastric mucosa remain unclear. A number of factors are important in mucosal defence including prostaglandins, the mucus-bicarbonate barrier, cellular regeneration, and blood flow.⁹⁻¹⁵ The mechanisms underlying NSAID mucosal damage are complex but include depletion of mucosal prostaglandins and the direct toxic effect of NSAIDs on epithelial cells.^{16,17} Studies on the process of adaptation have shown an increase in cellular regeneration, suggesting that this could represent one of the mechanisms underlying adaptation.^{18,19}

The process of adaptation has been well documented and has an interesting epidemiological parallel in that the risk of developing a complication associated with NSAID ingestion falls with continued ingestion, even to a level

associated with no increased risk after 10 prescriptions.²⁰

Epidermal growth factor (EGF) is a 53 amino acid polypeptide secreted in saliva and present in gastric juice.^{21,22} The salivary glands are normally the major source of gastric EGF,²³ and in rats sialoadenectomy is associated with decreased mucosal cytoprotection against a variety of noxious agents and a delay in healing in mucosal ulceration. The active component of saliva in this process has been shown to be EGF.²⁴⁻²⁷ It is well recognised that EGF is a potent mitogen, stimulating cellular regeneration and mucosal repair.²⁸ It also stimulates gastric mucus production²⁹ and suppresses acid secretion,³⁰ thereby providing a favourable environment for mucosal healing.

In this study the possible role of EGF in gastric mucosal adaptation to NSAIDs was assessed. Salivary and gastric EGF levels were measured in patients with arthritis before and after two weeks continuous treatment with NSAIDs.

Methods

Patients were studied in the context of a double blind randomised trial to assess the efficacy of a prostaglandin analogue, misoprostol (Searle), in the prevention of NSAID associated gastric lesions.

Patients attending the rheumatology clinic who were due to start NSAID treatment were invited to enter the study. None had a history of gastroduodenal ulceration and none had taken any aspirin or NSAIDs in the preceding three months. Informed consent was obtained and the study was approved by the Cambridge District Ethical Committee. Patients underwent an initial endoscopy. If this was normal, they were entered into the study and started on an NSAID of the rheumatologist's choice. They were then randomised to receive either misoprostol 200 µg three times daily or an identical placebo. After two weeks of continuous treatment, patients underwent a second endoscopy at which the mucosa was assessed for damage. At each endoscopy two biopsy specimens were taken for routine histological examination from macroscopically normal antral mucosa. All endoscopies were performed by one investigator (SMK), and mucosal damage was assessed by the method of Larkai.³¹

COLLECTION OF SAMPLES OF SALIVA

Samples of saliva were obtained from patients entering the study. Details of underlying disease, drugs, alcohol intake, and smoking habits were recorded. Patients were studied on the morning before the endoscopy, between 9.00 am and 1.00 pm after a two hour fast. Whole

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TABLE I Patient details, non-steroidal anti-inflammatory drug (NSAID) use, and endoscopic findings

	Placebo (n=10)	Misoprostol (n=5)
Age, mean (SEM) (y)	57.4 (3.9)	56.4 (5.98)
Type of arthritis:		
Rheumatoid	5	3
Osteoarthritis	4	2
Other	1	—
NSAIDs used:		
Diclofenac	6	3
Nabumetone	1	—
Indomethacin	1	—
Fenbufen	2	2
Flurbiprofen	2	—
Lesions at endoscopy	3	—

saliva was collected for 15 minutes without the use of any stimulant to increase flow. Patients were instructed not to swallow during the collection period, all saliva being expectorated. At the end of the 15 minute period, the volume was measured and an aliquot was frozen for EGF assay at a later date.

COLLECTION AND PREPARATION OF GASTRIC JUICE SAMPLES

Samples of gastric juice were obtained at each endoscopy. Gastric juice was aspirated down the suction channel of the endoscope into a trap. Care was taken to ensure the channel was clear of possible contaminating fluid before the sample was obtained. A 2 ml aliquot of gastric juice was obtained. A protonin (Trasylol) (0.1 ml) was added and the sample was frozen at -20°C for EGF analysis at a later date.

The concentration of EGF in gastric juice has been reported to be in the region of 0.3 ng/ml. As this was near the lower limit of detection of the radioimmunoassay available at the time of this study, aliquots of gastric juice were lyophilised to concentrate the samples. Two ml aliquots were initially titrated to pH 7.4 with 0.1 M NaOH. A known amount of 3H-Glucosamine was then added to enable corrections for recovery after lyophilisation to be made. Samples were then freeze-dried and reconstituted in 500 µl Tris buffer. Aliquots were taken for EGF assay and for amylase, which was measured to exclude gastroduodenal reflux. A further aliquot was taken for determination of 3H-Glucosamine, suspended in scintillation fluid, and counted in Beckman LA 1201 counter with automatic quenching. This enabled corrections to be made for recovery during lyophilisation. Values for recovery ranged from 62–90%.

ASSAY METHOD

Immunoreactive EGF was measured by radio-immunoassay (Biomedical Tech WA). Aliquots (100 µl) of human EGF standards or samples were mixed with rabbit anti-human EGF and

¹²⁵I-labelled human EGF. Samples were incubated at 4°C for 24 hours. Bound ligand was then precipitated by the addition of goat anti-rabbit IgG. After addition of buffer, samples were centrifuged at 2000 g for 15 minutes at 4°C. The supernatant was decanted and tubes were counted for two minutes in a gamma counter and EGF concentrations calculated against a standard curve. Inter-assay variation was 6% and intra-assay variation 4%.

Results were expressed as the mean (SEM). Statistical analyses were performed using a Student's *t* test when comparing values before and after NSAID ingestion within each group. Comparisons between groups were made using a Mann-Whitney U test as the groups were non-parametric.

Results

A total of 15 patients were entered into the study. Ten were randomised to receive placebo and five to receive misoprostol. None of these patients had evidence of sicca syndrome which can affect salivary EGF output.³²

ENDOSCOPIC APPEARANCES

Ten patients received placebo in addition to NSAIDs. Three developed erosions, but only one had symptoms. In the group of five patients taking misoprostol, none showed any evidence of mucosal damage. The patient details, NSAID use, and endoscopic appearances are summarised in Table I.

On initial examination mild chronic gastritis and *Helicobacter pylori* were found in four of the 10 placebo patients and two of five taking misoprostol. Only one patient who developed erosions was colonised with *H pylori*, and so this finding did not influence the results. There was no significant change on routine histological examination after NSAID ingestion.

SALIVARY EGF

There was no significant change in the salivary EGF concentration or output during the study (Table II). It is interesting to note, however, that in this relatively small group of patients, those who developed erosions tended to have lower salivary EGF concentrations on entry to the study. The group of patients with subsequent normal endoscopies had an initial salivary EGF concentration of 3.56 (0.64) ng/ml compared with 1.75 (0.54) ng/ml in those who developed erosions (p=NS).

GASTRIC JUICE EGF

In those patients taking misoprostol there was no significant change in the concentration of EGF in gastric juice: it was 0.33 (0.12) ng/ml initially and 0.32 (0.08) ng/ml after two weeks NSAID ingestion (p=NS). In the group of 10 patients taking placebo, however, the concentration of EGF in gastric juice increased from 0.42 (0.12) ng/ml to 0.69 (0.14) ng/ml (p<0.05) (Figure).

In the patients who developed erosions (n=3), the EGF concentration increased from 0.15

TABLE II Salivary epidermal growth factor (EGF) concentration and output in response to non-steroidal anti-inflammatory drug ingestion, values represent mean (SEM)

	Concentration (ng/ml)		Output (ng/15 min)	
	Before	After	Before	After
Placebo (n=10)	3.02 (0.54)	2.80 (0.41)	10.21 (1.60)	0.96 (1.72)
Misoprostol (n=5)	2.80 (0.76)	2.87 (0.73)	11.20 (5.35)	11.19 (4.57)

Gastric juice epidermal growth factor (EGF) concentrations before and two weeks after continued non-steroidal anti-inflammatory drug and placebo ingestion. Values represent mean (SEM).

(0.11) to 0.41 (0.06) ng/ml ($p < 0.05$). In the patients who had a normal second endoscopy ($n=7$), the EGF concentration rose from 0.53 (0.15) to 0.80 (0.19) ng/ml, a trend which does not reach statistical significance. Again, initial gastric EGF values tended to be lower in patients who subsequently developed erosions but this did not reach statistical significance (0.15 (0.15) v 0.53 (0.11) ng/ml ($p=0.15$)).

Gastroduodenal reflux of EGF secreted from Brunner's gland in the duodenum is unlikely to affect these findings as there was no significant change in amylase concentration in gastric juice samples (data not shown).

Discussion

This study shows an increase in gastric juice EGF concentrations in patients taking NSAIDs which could contribute to mucosal repair and adaptation. As there was no significant change in salivary EGF, which in normal circumstances is believed to be the main source of EGF in the stomach, and there was no evidence of increased duodenal reflux, the increased gastric EGF must come from elsewhere. It is unlikely to come from the blood as serum EGF concentrations are much lower than those of gastric juice and so mucosal damage and leakage of serum might be expected to reduce rather than increase gastric EGF. Another possibility is that it is produced in the stomach.

There are no reports showing mRNA for EGF in normal gastric mucosa.³³ Increased EGF in gastric epithelial cells has been shown in patients with gastritis³⁴ and it has therefore been suggested that EGF mRNA is present in gastric mucosa, albeit at minute levels.³³ It is therefore possible that gastric epithelial cells themselves may produce EGF in response to injury.

In this study, the increase in gastric EGF was more obvious in patients who developed erosions. Recently, induction of a novel EGF secreting cell lineage has been shown in gastric stem cells after injury.³⁵ This lineage secretes EGF onto the mucosal surface after mucosal damage. This could contribute to increased gastric EGF concentrations not only in patients with obvious erosions but also in those with endoscopically intact mucosa. Superficial erosions can be shown on microscopy of macroscopically normal mucosa in patients taking

NSAIDs,^{8,36} and so this mechanism may contribute even in those with endoscopically normal mucosa. The gastric mucosa, therefore, may respond to NSAID induced damage by EGF production, both from the epithelial cells and by the induction of stem cell lineages produced EGF. Further studies are needed to identify exactly where the increased EGF that we have demonstrated has come from. Morphological identification of EGF in the gastric mucosa, which we were technically unable to do in the present study, seems to be the logical way forward.

EGF in gastric juice has no effect on healthy intact mucosa as the EGF receptors are predominantly on the basal aspect of the epithelial cells.³⁷ Mucosal damage allows luminal EGF access to these receptors, however, and so initiates the mechanisms of repair such as mitogenesis³⁸ and also mucus production,²⁹ which helps both to provide a favourable environment for mucosal repair and contributes further to mucosal cytoprotection. As NSAIDs produce widespread superficial damage, luminal EGF could interact with basal EGF receptors and produce the increased cellular regeneration³⁹ observed in macroscopically normal mucosa in patients taking NSAIDs. In this manner superficial damage could be rapidly repaired and progression to frank mucosal ulceration could be prevented.

Factors other than EGF are also likely to contribute to the process of adaptation. Shorrock has shown a fall in mucosal blood flow after NSAID ingestion.⁴⁰ In that study adaptation was associated with a return to normal of blood flow. This is an important observation as mucosal repair mechanisms, such as those stimulated by EGF, cannot occur without adequate mucosal blood flow. Other growth factors such as transforming growth factor alpha (TGF) may also contribute. Increased gastric mucosal TGF has recently been shown in the rat gastric mucosa after acute injury.⁴¹ It also acts via the EGF receptor but whether it contributes to NSAID adaptation remains unknown.

It is widely accepted that EGF contributes to normal gastric mucosal cytoprotection. It is therefore an interesting observation in this study that those patients with low concentrations of salivary and gastric EGF on entry to the study seemed to be at an increased risk of developing mucosal damage after NSAID ingestion. Although in this small group this represented a trend only, it would be in accordance with numerous animal studies showing reduced gastric cytoprotection after sialoadenectomy, which leads to reduced gastric EGF concentrations.^{23,24} It has been suggested that patients with rheumatoid arthritis are at greater risk of NSAID induced gastric damage⁴² and these subjects have also been shown to have low salivary EGF concentrations.³² The suggestion that reduced salivary and gastric EGF concentrations render patients susceptible to NSAID induced damage fits with this hypothesis and supports the importance of EGF in mucosal defence. Further studies with larger numbers of subjects are needed to confirm that patients with low salivary and gastric EGF concentrations may

be at greater risk of developing gastric injury. These could represent a subset of patients who require protection by continued use of prostaglandin analogues such as misoprostol when NSAID treatment is indicated.

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