Non-steroidal anti-inflammatory drugs and prostaglandin effects on pepsinogen secretion by dispersed human peptic cells

A I Lanas, J Nerin, F Esteva, R Sáinz

Abstract
The effects of aspirin and ibuprofen on pepsinogen secretion were studied in isolated human peptic cells prepared from endoscopically obtained biopsy specimens after collagenase digestion, mechanical disruption, and percoll gradient centrifugation. Pharmacological concentrations of aspirin and ibuprofen (10^{-8}-10^{-4} M), potentiated histamine (10^{-5}-10^{-4} M) and forskolin (10^{-5} M) stimulated pepsinogen secretion without affecting basal secretion, acetylcholine (10^{-6} M) stimulated pepsinogen secretion or cell vitality. Augmentation of secretagogue stimulated pepsinogen secretion was dependent on extracellular calcium because potentiation was abolished by calcium depletion of the medium. Cimetidine inhibited the potentiation effect on histamine but not on forskolin stimulated pepsinogen secretion, thus suggesting that this augmentation was independent of histamine H$_2$ receptors. Of interest, potentiation was also independent of endogenous prostaglandin inhibition because exogenous addition of prostaglandin E$_3$ and D$_3$ increased both basal and acetylcholine stimulated pepsinogen secretion in a dose dependent way, but they did not modify histamine or histamine plus aspirin or ibuprofen stimulated pepsinogen secretion. In conclusion, aspirin and ibuprofen potentiate secretagogue stimulated pepsinogen secretion by dispersed human peptic cells and this might be an additional mechanism of non-steroidal anti-inflammatory drug (NSAID) induced gastric injury. This potentiation effect is regulated by calcium, independent of endogenous prostaglandin inhibition and seems to act on pepsinogen secretion at a post-receptor site.

It is widely accepted that non-steroidal anti-inflammatory drug (NSAID) use is associated with a high prevalence of gastroduodenal injury. It is also recognised that the presence of acid and pepsin enhances the potential damaging effect of these drugs and, therefore, anti-secretory therapy is commonly prescribed to treat or prevent damage induced by NSAIDs. The pathogenic mechanisms of NSAID induced gastroduodenal damage remains unclear, although different mechanisms, most of them related to prostaglandin inhibition, have been proposed.

Aspirin and other non-salicylate NSAIDs have been reported to increase or potentiate histamine stimulated acid secretion in vivo and in vitro in different animal models, suggesting that enhanced gastric acid secretion could play a part in the pathogenesis of NSAID induced gastroduodenal mucosal damage. The mechanisms of NSAID stimulated acid secretion have been extensively explored by Levine et al in an in vitro rabbit parietal cell model, suggesting that NSAID induced acid secretion is regulated by the presence of calcium and the inhibition of prostaglandins.

Although available information provides sufficient evidence that intramucosal activation of acid proteases, mainly pepsinogens, plays an important part in peptic diseases of the upper gastrointestinal tract, there are almost no data on the potential effects of NSAIDs on pepsinogen secretion by the stomach. In this study we have examined in vitro the effects of two widely and more commonly used NSAIDs (aspirin and ibuprofen) on pepsinogen secretion by dispersed human peptic cells obtained from endoscopic biopsy specimens.

Methods

Chemicals
Chemicals were obtained from the following sources: bovine serum albumin (fraction V), acetylcholine, histamine, cimetidine, aspirin, ibuprofen, forskolin, HEPES, percoll, crystalline porcine pepsinogen, soybean trypsin inhibitor, ethylene glycol tetracetic acid (EGTA), and glucose from Sigma Chemical (St Louis, MO). Collagenase (type IV) from Worthington Biochemical (Freehold, NJ); bovine haemoglobin from Gibco Diagnostics (Madison, WI); and prostaglandin E$_2$ and D$_2$ from Calbiochem (La Jolla, CA).
69 patients undergoing upper gastrointestinal endoscopy (45 men and 15 women). The mean (SEM) age of this population was 59.11 (1.59) (18–61). In 20 of these patients the endoscopy was normal, 18 had an active duodenal ulcer, 14 a healed duodenal ulcer, nine a hiatal hernia, six oesophagitis, five duodenitis, five antritis, and two a gastric ulcer. Most of them (55 of 69) were or had recently been receiving H2 receptor antagonists, and the rest were receiving antacids or were free of any treatment. Helicobacter pylori status in the corpus of the stomach was not determined, but it was in the antrum (CLO test) in 43 of 69 patients (positive in 40). The study was approved by the Institutional Review Board and written informed consent was obtained from all patients.

Cell isolation

The method used to isolate peptic cells had been previously described elsewhere. In brief, eight to 10 endoscopically obtained gastric biopsy specimens (jumbo forceps) from the oxyntic area were transported in Ringer’s solution that had been gassed with 95% O2 and 5% CO2. The specimens then received a 20 minute digestion in 0.1% collagenase solution with 0.2% trypsin inhibitor in a shaking water bath at 37°C. This initial collagenase solution was discarded and replaced and digestion continued for 45 more minutes. The cells and glands were collected by centrifugation, resuspended in Ringer’s solution without calcium and magnesium, and subjected to repetitive pipetting for several minutes. The cells were filtered through 100 µm Teflon mesh and resuspended in Ringer’s solution containing calcium and magnesium. The cells were then taken up in 1 ml of Ringer’s solution and layered onto 2 ml 50% (v/v) percoll containing isotonic HEPES buffered Ringer’s solution, which contained 82.2 mM NaCl, 4.0 mM KCl, 1.8 mM CaCl2, 0.8 mM MgCl2, 17.8 mM NaH2PO4, 0.8 mM NaHCO3, 11.5 mM glucose, and 0.2% bovine serum albumin. The cells were then centrifuged at 14 000 g for 20 minutes at 4°C (Eppendorf Centrifuge, Model 5412). After this centrifugation the bottom of the gradient containing mainly red blood cells was discarded, and the remaining cells were washed and layered again onto 2 ml 33% (v/v) containing isotonic HEPES buffered Ringer’s solution. After another centrifugation at 14 000 g for 10 minutes, the top of the gradient containing mainly the parietal cells was discarded and a clearly different layer at the bottom contained the chief cells. The isolated chief cells were greater than 90% pure by microscopy (haematosylin and eosin staining procedure and periodic acid Schiff reaction), and >90% viable by trypan blue exclusion and >89% by flow cytometry (Epics Elite, Coulter, Hialeah Fl, USA) using the fluorochromes, rhodamine 123 and propidium iodide to measure live and dead cells respectively. The pepsinogen content of this layer was 100 times higher than the pepsinogen obtained from the top layer containing mainly

Unless otherwise stated, the standard Ringer solution contained 82.2 mM NaCl, 4.0 mM KCl, 1.8 mM CaCl2, 0.8 mM MgCl2, 17.8 mM NaH2PO4, 0.8 mM NaHCO3, 11.5 mM glucose, and 0.2% bovine serum albumin. The medium was equilibrated with 95% O2–5% CO2 and the pH was adjusted to 7.25. Ca2+ low medium contained 0.1 mM CaCl2 and no MgCl2, and Ca free medium contained no CaCl2 or MgCl2.

Biopsy specimens

Gastric biopsy specimens were obtained from

Figure 1: Effects of different concentrations of both aspirin (ASA) and ibuprofen (IBU) on two different concentrations of histamine (HIS) stimulated pepsinogen secretion from dispersed human peptic cells. Basal secretion in these experiments was 4.86 (0.57)% of total/30 min. n=4–7. *p<0.05 with respect to histamine stimulated secretion, †p<0.05 with respect to basal secretion.

Figure 2: Effects of both aspirin (ASA) (10^-6 M) and ibuprofen (IBU) (10^-4 M) on forskolin (FK) (10^-7 M) stimulated pepsinogen secretion from dispersed human peptic cells. Removal of extracellular calcium from the medium suppressed the potentiation effect of both drugs on forskolin stimulated pepsinogen secretion. Basal secretion in these experiments was 3.40 (0.29)% of total/30 min. n=4–5. *p<0.05 with respect to forskolin.

82.2 mM NaCl, 4.0 mM KCl, 1.8 mM CaCl2, 0.8 mM MgCl2, 17.8 mM NaH2PO4, 0.8 mM NaHCO3, 11.5 mM glucose, and 0.2% bovine serum albumin.

The medium was equilibrated with 95% O2–5% CO2 and pH was adjusted to 7.25. Ca2+ low medium contained 0.1 mM CaCl2 and no MgCl2, and Ca free medium contained no CaCl2 or MgCl2.
Non-steroidal anti-inflammatory drugs and prostaglandin effects on pepsinogen secretion by dispersed human pepsic cells

Figure 3: Effects of cimetidine (CIM) (2×10⁻⁶ M) on aspirin (10⁻⁶ M) potentiation of either histamine (HIS) (10⁻⁴ M) or forskolin (FK) (10⁻⁵ M) stimulated pepsinogen secretion from dispersed human pepsic cells. Results are expressed as percentage of pepsinogen secretion obtained after stimulation with either histamine or forskolin minus basal secretion and set at 100%. Basal secretion for both histamine and forskolin was similar to those values expressed in previous figures. n=3-5, *p<0.05.

Ringer’s solution and counted (haematocytometer). Aliquots of 10^5 cells were then placed in 1.5 ml polypropylene test tubes containing the appropriate agents and incubated in a shaking water bath under an atmosphere of 95% O₂-5% CO₂ at 37°C for various times. In general, cells from a single patient were sufficient for 9-11 aliquots (including duplicates). If the experiment was high in number of aliquots or samples, two sets of cells from two different subjects were put together. In this case n=4 means eight people. The experiments were finished by centrifuging the tubes for 17 seconds at 14 000 g and pipetting off the supernatants. An aliquot of the supernatant was assayed for pepsinogen activity using a modified Anson-Mirsky method using acid denaturated haemoglobin as the substrate as previously described.16 The mixture was incubated at 37°C for five hours and the reaction stopped by adding 1 ml of 7% trichloroacetic acid solution followed by centrifugation at 13 000 rpm for five minutes. The optical absorbance of the supernatant was read at 280 nm. Pepsinogen released into the medium was expressed as percentage of the total pepsinogen initially present in the cells. Secretory responses to stimuli were calculated in the output per unit time with basal output subtracted. The mean (SEM) basal rate of pepsinogen secretion in the experiments was 0.18 (0.01)% of total pepsinogen content per minute. The concentrations of aspirin and ibuprofen used in these experiments are within the range that can be expected in patients who take these drugs when prescribed to treat rheumatological conditions,17 18 and they do not modify optical absorbance when measuring pepsinogen activity.

In some experiments, carried out to study the role of calcium in NSAIDs on pepsinogen secretion, cells were washed once in calcium free Ringer’s solution, resuspended in calcium free Ringer’s solution containing 0.5 mM EDTA for three minutes to remove extracellular Ca²⁺, washed again, and finally resuspended in calcium free Ringer’s solution plus the drugs to be tested.

Cell labelling and detection of eicosanoid production

Synthesis of prostaglandins from endogenous cellular sources was measured by radiolabelling resuspended cells with 10 µM 1-¹⁴C-arachidonic acid (New England Nuclear, Daimlerstrasse, Germany) for 30 minutes. Cells were resuspended in standard Ringer’s solution with 0.2% essentially fatty acid free bovine serum albumin. After incubation, the supernatant (1 ml) was acidified with 1 M citric acid to pH 3.0 and injected into C₁₈ cartridges (Sep-Pack, Millipore Iberica, Madrid) containing octadecylsil silica. Elution and detection of arachidonic acid metabolites have been described elsewhere.19 In brief, the cartridges were first washed with 3 ml of acified water (pH 3.0) and 3 ml of water, then with 1 ml of hexane for partial elution of non-polar lipids, and finally with 2 ml of ethanol.

Figure 4: Effects of both aspirin (ASA) and ibuprofen (IBU) on acetylcholine (Ach) stimulated pepsinogen secretion from dispersed human pepsic cells. Basal secretion in these experiments was 5.24 (0.27)% of total/30 min. n=3-4.

Experiments and calculations

The warmed cells were resuspended in fresh parietal cells. No significant pepsinogen secretion was obtained from the top layer when stimulated with acetylcholine or cholecystokinin octapeptide.

Eight biopsy specimens from one young patient yielded about 10⁶ cells that were suspended in Ringer’s solution under 95% O₂-5% CO₂ atmosphere and refrigerated overnight. The next morning, the cells were allowed to warm at room temperature before they were used in experiments. No experiments were performed if cell viability was lower than 89% and experiments with low total pepsinogen content were discarded.
Results

Effects of aspirin and ibuprofen on pepsinogen secretion
Neither aspirin nor ibuprofen had any significant effect on basal pepsinogen secretion (Table), but both drugs potentiated maximal (10⁻⁴ M) histamine stimulated pepsinogen secretion in a dose dependent way (Fig 1). Maximal stimulation was obtained with 10⁻⁴ M aspirin (2.96 ± 0.38)%/30 min and 10⁻⁶ M ibuprofen (3.08 ± 0.56)%/30 min. Cell viability was not affected by these concentrations as shown by both trypan blue exclusions and the fluorochromes rhodamine 123 and propidium iodide (89-9 (2.7)% of viable cells in controls vs 90.8 (2.5)% with 10⁻⁴ M aspirin and 90.8 (2.7)% with 10⁻⁶ M ibuprofen; n = 5). Potentiation of pepsinogen secretion was also seen when peptic cells were pre-stimulated with lower doses of histamine (10⁻⁵ M) and reinforces the physiological significance of these effects. Like histamine, pepsinogen secretion stimulated by forskolin (10⁻⁵ M), an intracellular secretagogue that activates the catalytic subunit of adenylate cyclase, was also potentiated by both aspirin and ibuprofen (Fig 2). Cimetidine (2×10⁻⁴ M) blocked aspirin potentiation of histamine stimulated pepsinogen secretion but did not inhibit significantly aspirin effects on forskolin stimulated pepsinogen secretion (Fig 3), showing that cimetidine inhibited histamine stimulation in itself rather than the potentiation of aspirin.

Aspirin and ibuprofen (10⁻⁴–10⁻⁵ M) failed to potentiate acetylcholine stimulated pepsinogen secretion suggesting that the potentiating effect of these two drugs may be mediated by mechanisms that share some intracellular pathway (for example, Ca⁺⁺) but do not change muscarinic related responses (Fig 4).

Role of calcium in aspirin and ibuprofen potentiation of pepsinogen secretion
Aspirin and ibuprofen potentiate histamine and forskolin but not acetylcholine stimulated pepsinogen secretion showing that these drugs may activate mechanisms of action different from those used by histamine or forskolin and similar to those used by acetylcholine. To study whether such mechanisms were calcium dependent, cells were deprived of extracellular calcium and then stimulated. Under these conditions aspirin and ibuprofen completely failed to potentiate either histamine or forskolin stimulated pepsinogen secretion (Figs 2 and 5).

Role of prostaglandins in aspirin and ibuprofen potentiation of pepsinogen secretion
NSAIDs inhibit prostaglandin secretion in gastric cells and prostaglandins inhibit gastric acid secretion in vivo and in vitro. This could be one mechanism to explain the potentiation effects of NSAIDs on pepsinogen secretion. In our system, we have seen that the addition of 10⁻⁴ M aspirin inhibited...
Non-steroidal anti-inflammatory drugs and prostaglandin effects on pepsinogen secretion by dispersed human pepsic cells

endogenous production of both radiolabelled PGE$_2$ and PGD$_2$ by peptic cells (PGE$_2$: 3930 (135) cpm/µg protein v 1115 (236)* with aspirin; PGD$_2$: 4194 (486) cpm/µg protein v 1483 (596)* with aspirin; n=3-5; *p<0.05). In contrast with published data, we found that exogenous PGE$_2$ and PGD$_2$ both induced a dose dependent increase in pepsinogen secretion in human peptic cells (Fig 6). Maximal secretion was obtained at $10^{-7}$ M PGE$_2$ (3-30 (1-11)%/30 min) and $10^{-8}$ M with PGD$_2$ (2-51 (0-8)%/30 min). Pepsinogen secretion induced by prostaglandins was in addition to that induced by acetylcholine. However, prostaglandins failed to increase histamine stimulated pepsinogen secretion (Fig 7) and did not modify aspirin and ibuprofen potentiation of histamine stimulated pepsinogen secretion either (Fig 8).

Discussion

Aspirin and other NSAIDs have been reported to increase basal or histamine stimulated acid secretion, or both in vivo and also in vitro in humans and different animal models. These effects have suggested that enhanced gastric secretion by NSAIDs could be a factor in the pathogenesis of NSAID induced gastroduodenal injury. Species differences have been reported on the actions of NSAIDs in rabbits, frogs, and humans on parietal cells and show that data from one species cannot be fully extrapolated to others (for example, humans).

Unlike gastric acid secretion, there are almost no data on the potential effects of NSAIDs on pepsinogen secretion. A report from Ohe et al showed that aspirin increased the ratio of alkali-labile to total pepsinogen in the homogenates of gastric mucosa in rats and considered that activated pepsinogen was an essential step in ulcer formation by the hydrogen ion back diffusion. By using a 'in vitro' model of isolated human peptic cells, we now provide evidence for the first time that both aspirin and ibuprofen (two widely used NSAIDs) potentiate secretagogue stimulated pepsinogen secretion in humans. The potentiation of histamine stimulated pepsinogen secretion was seen at concentrations of both aspirin and ibuprofen that are commonly reached in plasma of patients who regularly used these drugs. Furthermore, the potentiation effect was also seen on different histamine concentrations ($10^{-4}$-$10^{-6}$ M) that are in the range required for maximum acid stimulated in the vascularly perfused rat stomach.

We have also explored the mechanisms by which aspirin and ibuprofen potentiate stimulated pepsinogen secretion by human peptic cells. Receptor and signal transduction pathway studies have clearly defined two sets of pepsinogen secretagogues: (a) those that activate adenyl cyclase (for example, histamine, forskolin, vasoactive intestinal peptide, etc) and stimulate intracellular cAMP and (b) calcium mediated secretagogues (for example, acetylcholine, cholecystokinin, gastrin, etc); these agents interact with receptors in chief cells and result in the activation of enzymes that mediate phospholipid hydrolysis, release of calcium from intracellular stores, and the activation of calcium-calmodulin dependent protein kinases. Aspirin and ibuprofen did not seem to potentiate pepsinogen secretion by affecting the adenylate cyclase/cAMP system or the H$_2$ receptor. Cimetidine inhibited the potentiation of histamine stimulated pepsinogen secretion by these drugs but failed to block the potentiation effect on forskolin stimulated pepsinogen secretion. Because forskolin activates the catalytic subunit of adenylate cyclase intracellularly, these results suggest a regulatory role for aspirin and ibuprofen in conjunction with secretagogues distal to the site of the catalytic subunit of adenylate cyclase activation. On the other hand, aspirin and ibuprofen effects on pepsinogen secretion seem dependent on extracellular calcium, as these two drugs failed to potentiate either histamine or forskolin stimulated pepsinogen secretion when extracellular calcium was removed from the media.

Figure 7: Prostaglandin (PGE$_2$) stimulation of pepsinogen secretion from dispersed human pepsic cells is in addition to that obtained with acetylcholine (Ach), but it is not to pepsinogen secretion obtained with histamine (HIS) stimulation. Basal secretion in these experiments was 5-75 (1-1)% of total/30 min. n=4, *p<0.05, tp<0.01.

Figure 8: Exogenous prostaglandin (PGE$_2$) addition does not affect aspirin (ASA) and ibuprofen (IBU) potentiation of histamine (HIS) stimulation of pepsinogen secretion from dispersed human pepsic cells. Basal secretion in these experiments was 4-80 (0-60)% of total/30 min. n=3.
In rabbit parietal cells aspirin also potentiates histamine, forskolin, and dbcAMP stimulated aminopyrine uptake by calcium dependent mechanisms. In such a system aspirin increases intracellular calcium from intracellular sources, although an effect of aspirin to increase intracellular Ca$$^{2+}$$ stores from the extracellular space was not excluded. Prolonged EGTA washes deplete both intracellular and extracellular Ca$$^{2+}$$ and a brief EGTA wash only depletes extracellular Ca$$^{2+}$$.

We have confirmed this in histamine stimulated pepsinogen secretion. This suggests that extracellular calcium is needed for aspirin and ibuprofen to potentiate pepsinogen secretion in human peptic cells probably by refilling intracellular calcium stores. Furthermore, both aspirin and ibuprofen failed to potentiate acetylcholine stimulated pepsinogen secretion, a natural calcium dependent secretagogue. It is possible that intracellular calcium mobilisation by acetylcholine is potent enough to prevent further calcium increase by NSAIDs.

By finding that exogenous prostaglandins actually stimulated peptic cells, we ruled out a prostaglandin mechanism by which aspirin and other NSAIDs might potentiate pepsinogen secretion. Furthermore, this increase was in addition to that produced by acetylcholine suggesting that prostaglandins and acetylcholine act through independent pathways. Previous reports had already shown that pepsinogen effects on chief cells from dogs and guinea pigs were mediated by cAMP. We have confirmed this in our system because the exogenous addition of prostaglandin E2 to histamine stimulated pepsinogen secretion was not different to that obtained with histamine alone. Furthermore, aspirin potentiation of histamine stimulated pepsinogen secretion was neither inhibited nor potentiated by the addition of exogenous prostaglandin E2.

By using patient material it can be questioned whether longer term drug use may disturb the responsiveness of the mucosa (for example, H2 receptor antagonists used in most of our patients). However, we think that such a possibility does not invalidate our results. As in most studies of isolated peptic cells, in this work each single experiment had its own control samples and responses to different stimuli are always expressed as a reference to the values obtained in such control samples. Therefore, if the basal state of peptic cells was changed by longer term drug use, such a difference is eliminated by expressing the results as percentage of total pepsinogen content and basal subtracted. Furthermore, because most of our patients were receiving H2 blockers, the potential effects of these drugs on the responsiveness of the mucosa (increase or decrease secretory calcium, etc) were present in all samples of a particular experiment and probably did not affect the main effect of the drug tested (NSAID, prostaglandins, etc) in our experiments (potentiation, inhibition, etc). Moreover, our results show that the effects of both NSAIDs and prostaglandins on pepsinogen secretion are independent of H2 receptors. Finally, the process of isolating cells includes repeated washing of cells with fresh Kreb's Ringer solution (at least 10 times), which eliminates the potential presence of any drug taken by the patient before the endoscopy.

Our results show also some variability in basal pepsinogen secretion in the different experiments. Studies on pepsinogen secretion in experimental conditions always show some interassay variability, but this has been found to be greater in isolated human peptic cells. The reasons for this are not known, but it may depend, at least in part, on different factors such as the different age of the subjects used in the study, the presence of a peptic ulcer disease, the presence of Helicobacter pylori infection, etc. In this way, serum pepsinogen has been found to decrease in ulcer patients after H pylori eradication and in vitro sonication of cultured cells. It is possible that intracellular calcium mobilisation by acetylcholine is potent enough to prevent further calcium increase by NSAIDs.

Whether H pylori affects pepsinogen secretion of human peptic cells, or the response of these cells to natural agonists (acetylcholine, histamine, prostaglandins), and whether this infection or any other factor are responsible for the variability in basal pepsinogen secretion from isolated human peptic cells warrant further studies.

In conclusion, our data show that pharmacological doses of two of the most widely used NSAIDs (aspirin and ibuprofen) potentiate secretagogues stimulated pepsinogen secretion from dispersed human peptic cells and this might be an additional mechanism of NSAID induced gastric mucosal injury in humans. The potentiation effect of these drugs is calcium dependent, independent of endogenous prostaglandin inhibition, and may be mediated in peptic cells at a post-receptor site.

This study was supported by a grant from the Asociación para Investigaciones Gastroenterológicas de la Provincia de Zaragoza. The authors thank Professor Basíl I Hirschowitz for critical discussion and review of the manuscript.

Non-steroidal anti-inflammatory drugs and prostaglandin effects on pepsinogen secretion by dispersed human peptic cells


Non-steroidal anti-inflammatory drugs and prostaglandin effects on pepsinogen secretion by dispersed human peptic cells.

A I Lanas, J Nerín, F Esteva and R Sáinz

Gut 1995 36: 657-663
doi: 10.1136/gut.36.5.657

Updated information and services can be found at:
http://gut.bmj.com/content/36/5/657

Email alerting service

These include:
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections

Articles on similar topics can be found in the following collections

Gastrointestinal hormones (848)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/