Inhibition of gastric acid secretion by nimesulide: A possible factor in its gastric tolerability

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ABSTRACT

Objective
To study the effect of nimesulide on acid secretion in mouse isolated stomach.

Methods
Isolated lumen-perfused mouse stomachs were monitored by pH-electrodes. Gastric acid secretion was stimulated with histamine or 5-methylfurmethide (5-MeF, a stable acetylcholine derivative), and the effect of nimesulide and indomethacin were studied alone and in combination with famotidine.

Results
The concentration-dependent stimulation of gastric acid output by histamine (Hill equation fitting parameters: log[A]_50, 5.44 ± 0.15; p, 1.01 ± 0.11; , 0.64 ± 0.05) was inhibited by famotidine, and also by nimesulide (log r = 0.79 ± 0.10 at 30 M). However, nimesulide also reduced the maximum acid output. The shift produced by nimesulide and famotidine in combination indicated a greater than additive effect, suggesting that nimesulide was not acting at histamine H2-receptors (Shankley et al., 1988) (2). Indomethacin reduced acid secretion only at the highest concentration (100 M). Furthermore, the histamine-receptor-independent stimulation of gastric acid output by 5-MeF was greatly inhibited by nimesulide, which also suggests that nimesulide was acting on the parietal cell signaling pathway at a non-histamine-receptor site.

Conclusion
The relatively low risk of gastric mucosal damage with nimesulide is thought to involve its weak inhibition of gastric prostaglandin synthesis and its weak acidity, but another factor might be the ability to reduce gastric acid production. However, the effect of nimesulide on human gastric acid secretion remains to be investigated.

Introduction
Nimesulide has been shown in clinical studies with volunteers or patients with osteoarthritis to cause less gastric damage than other non-steroidal anti-inflammatory drugs (NSAIDs) (3, 4). In fresh human gastric mucosa, which contains primarily constitutive cyclooxygenase-1 (COX-1), nimesulide was less potent than indomethacin in reducing prostaglandin synthesis (5). Nimesulide preferentially inhibits inducible cyclooxygenase-2 (COX-2) in whole blood and purified enzymes (5-7). In addition, nimesulide has various other anti-inflammatory properties (8), including inhibition of the action and release of histamine (9). Since histamine plays an essential role in gastric acid secretion, in this study we have investigated the effect of nimesulide on stimulated acid secretion in mouse isolated stomach. Acid secretion was stimulated with histamine or the stable acetylcholine derivative 5-methylfurmethide (5-MeF), and the effects of nimesulide and indomethacin alone and in combination with the histamine-2 receptor antagonist famotidine were studied.

Methods
Acid secretion from lumen-perfused mouse isolated stomachs was monitored by pH-electrodes (1). Male C57 BL6 mice weighing 25-28 g, maintained under conditions specified by British Home Office Regulations, were deprived of food 16 hrs prior to experimentation but had free access to water. After killing by cervical dislocation, the distal oesophagus was ligated and the stomach removed. One cannula was inserted into the pylorus via the duodenal bulb, and another cannula was tied into the fundus via a small incision. The stomach was flushed through with unbuffered mucosal solution (mM: NaCl 135, KCl 4.8, MgSO4 1.2, CaCl2 0.65, glucose 31.6), and transferred to an organ bath containing 40 ml buffer-
ed serosal solution (mM: NaCl 118, KCl 4.8, MgSO₄ 1.2, KH₂PO₄ 1.14, NaHPO₄ 15.9, CaCl₂ 1.3, glucose 31.6) at 37°C and gassed with 5% CO₂ in O₂. The preparations were perfused continuously (1 ml/min) via the fundic cannula and out through the pyloric cannula with unbuffered mucosal solution at 37°C gassed with 100% O₂. The effluent passed over a pH-electrode system set at 12 cm above the preparation to distend the stomach. All drugs (volume not exceeding 1 ml) were added to the 40 ml serosal solution to obtain a single administration of histamine.

Histamine E/A curves were obtained in the absence (p[A₅₀] = 5.64 ± 0.06) and the presence of 20 M nimesulide (p[A₅₀]₀ = 5.23 ± 0.09) or 0.15 M famotidine (p[A₅₀]₀ = 4.80 ± 0.03), or both (p[A₅₀]₀ = 4.35 ± 0.04) (Fig. 2).

When these data were inserted into equations 2 and 3, the S₅₀ value but not the Sₐ value was significantly different from zero, indicating a multiplicative rather than an additive effect, and suggesting that nimesulide was not acting at a histamine H₂-receptor site.

5-MeF, in the presence of famotidine 30 (M to block H₂ receptors), produced a concentration-dependent stimulation of gastric acid output in the mouse stomach preparation (p[A₅₀]₀ = 7.35; p, 3.38; 0.56, n = 12, using mean data since not all the individual E/A curves fitted the Hill equation).

Nimesulide concentration-dependently

Data analysis
Concentration-effect curve data from individual preparations were fitted by means of an interactive least squares minimisation program to the Hill equation:

\[
E = \frac{[A]^p}{[A]_{SO}^p + [A]^p}
\]

to provide estimates of the midpoint location (p[A₅₀]), midpoint slope parameter (p) and the maximum asymptote ( ), as described by Black & Shankley (1). Computed curve-fitting parameter estimates were compared by one-way ANOVA.

Combined dose ratio (r) analysis was performed as previously described (10). In brief, the theoretical relationships which describe the syntopic (additive, \( r_{B+C} = r_B + r_C - 1 \)) and the allo-topic (multiplicative, \( r_{B+C} = r_B \times r_C \)) interaction between two antagonists were expressed as test statistics (S) in terms of the experimentally estimable log[A₅₀] values:

\[
S_A = \log[A_{SO}]_{B+C} - \log([A_{SO}]_B + [A_{SO}]_C - [A_{SO}])
\]

\[
S_M = \log[A_{SO}]_{B+C} - \log[A_{SO}]_B - \log[A_{SO}]_C + \log[A_{SO}]
\]

Test statistical values of zero are expected for model compliance.

Results
The concentration-dependent stimulation of gastric acid output by histamine (Hill equation fitting parameters: p[A₅₀]₀ = 5.44 ± 0.15; p, 101 ± 0.11; 0.64 ± 0.05, n = 5) was concentration-dependently shifted rightward by famotidine with an estimated pA₂ value of 7.55 ± 0.11, consistent with an action on histamine H₂ receptors. Nimesulide produced a rightward shift of the histamine E/A curve (log r = 0.79 ± 0.10 at 30 M), as shown in Figure 1. However, unlike famotidine, nimesulide also reduced the maximum response (> 90% at 100 M). Indomethacin reduced the histamine-stimulated acid secretion only at the highest concentration (100 M), producing a curve shift (log r = 0.61 ± 0.12) and a reduction of secretion (P < 0.05). Histamine E/A curves were obtained in the absence (p[A₅₀]₀ = 5.64 ± 0.06) and the presence of 20 M nimesulide (p[A₅₀]₀ = 5.23 ± 0.09) or 0.15 M famotidine (p[A₅₀]₀ = 4.80 ± 0.03), or both (p[A₅₀]₀ = 4.35 ± 0.04) (Fig. 2).

Nimesulide concentration-dependently

Fig. 1. Acid secretion in mouse isolated stomach stimulated with histamine in the absence and presence of nimesulide.

Fig. 2. Acid secretion in mouse isolated stomach stimulated with histamine in the absence and presence of nimesulide and famotidine.
shifted the 5-MeF curve to the right and reduced the maximal acid secretion, as shown in Figure 3 (log r = 0.40 with 10 M nimesulide).

Discussion
Gastric acid secretion is regulated by central and peripheral pathways that involve endocrine, neurocrine and paracrine mechanisms. Histamine released from enterochromaffin-like cells that act in a paracrine fashion, binds to H2 receptors on parietal cells to activate adenylate cyclase, and the resultant increase in cAMP leads to acid secretion. The vagus regulates acid secretion both by releasing acetylcholine that acts on parietal cells, and by stimulating histamine release. Prostaglandin E2 on the other hand acts on the parietal cells to inhibit adenylate cyclase and so decrease acid production (11).

In our study, histamine-stimulated acid secretion in mouse isolated stomach was concentration-dependently inhibited by nimesulide. This occurred without the involvement of H2 receptors, as demonstrated by the effects of nimesulide and famotidine alone and in combination. Furthermore, the histamine-receptor-independent stimulation of gastric acid output by 5-MeF in the presence of famotidine was also inhibited by nimesulide, consistent with nimesulide acting on the parietal cell signaling pathway at a non-histamine-receptor site.

Reduction of gastric acid in the stomach by nimesulide might therefore be one of the contributory factors that account for its relatively small amount of mucosal damage. However, this conclusion is based on results in mouse stomachs, and the effect of nimesulide on human gastric acid secretion remains to be investigated.

References