NSAIDs counteract *H. pylori* VacA toxin-induced cell vacuolation in MKN 28 gastric mucosal cells

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Ricci, Vittorio, Barbara A. Manzo, Concetta Tuccillo, Patrice Boquet, Ulderico Ventura, Marco Romano, and Raffaele Zarrilli. NSAIDs counteract *H. pylori* VacA toxin-induced cell vacuolation in MKN 28 gastric mucosal cells. *Am J Physiol Gastrointest Liver Physiol* 283: G511–G520, 2002; 10.1152/ajpgi.00046.2002.—The relationship between non-steroidal anti-inflammatory drugs (NSAIDs) and *Helicobacter pylori*-induced gastric mucosal injury is still under debate. VacA toxin is an important *H. pylori* virulence factor that causes cytoplasmic vacuolation in cultured cells. Whether and how NSAIDs affect VacA-induced cytotoxicity is unclear. This study was designed to evaluate the effect of NSAIDs on *H. pylori* VacA toxin-induced cell vacuolation in human gastric mucosal cells in culture (MKN 28 cell line). Our data show that 1) NSAIDs (indomethacin, aspirin, and NS-398) inhibit VacA-induced cell vacuolation independently of inhibition of cell proliferation and prostaglandin synthesis; 2) NSAIDs impair vacuole development/maintenance without affecting cell binding and internalization of VacA; and 3) NSAIDs, as well as the chloride channel blocker 5-nitro-2-(3-phenylpropylamino) benzoic acid, also inhibit cell vacuolation induced by ammonia. We thus hypothesize that NSAIDs might protect MKN 28 cells against VacA-induced cytotoxicity by inhibiting VacA channel activity required for vacuole formation.

aspirin; chloride channel blockers; cyclooxygenases; indomethacin; NS-398

**HELCOBACTER PYLORI** (*H. pylori*) and nonsteroidal anti-inflammatory drugs (NSAIDs) are recognized to cause the large majority of peptic ulcers through apparently different pathological mechanisms (15, 26).

*H. pylori*-related gastric mucosal injury depends on the inflammatory response of the host and on the release of virulence factors, among which VacA toxin plays a central role (3, 38, 43, 59). VacA is a protein toxin, formed by monomers of ~90 kDa, able to induce cytoplasmic vacuoles in eukaryotic cells in culture (6, 40). Cytoplasmic vacuoles are also present in vivo in the gastric epithelium of *H. pylori*-colonized patients (10, 11, 53). When given to mice, purified VacA causes gastric epithelial damage closely resembling that found in *H. pylori*-colonized patients (50). After cell internalization, VacA localizes in the endocytic-endosomal compartment from which vacuoles originate (36, 41, 42). Vacuole development is strictly dependent on the presence in the incubation medium of weak bases like ammonia (which can be generated by *H. pylori* urease; see Refs. 9 and 42). Recently, it has been reported that VacA may act as a channel-forming toxin, and it has also been proposed that VacA channels play a direct role in cell vacuolation. Endocytosed VacA channels could stimulate the turnover of endosomal vacuolar-type H+-ATPase (V-ATPase) by increasing the permeability of the endosomal membrane to anions (for reviews, see Refs. 40 and 43). This would lead to the accumulation of osmotically active species causing an osmotic imbalance of late endosomes with subsequent vacuole formation.

In patients consuming NSAIDs, abrogation of processes such as maintenance of adequate gastric muco-
sal blood flow or mucus/bicarbonate secretion, which largely depends on constitutive PG synthesis, plays a key role in the pathogenesis of gastric mucosal injury (15, 55). On the other hand, *H. pylori* injury to the gastric mucosa occurs despite stimulation of PG synthesis (19, 46).

The relationship between *H. pylori* and NSAIDs in terms of their effects on gastric mucosa remains controversial. Some clinical studies have reported no interaction between *H. pylori* and NSAIDs or a protective role of *H. pylori* in patients given NSAIDs (16, 21, 25). Other studies have shown harmful effects of *H. pylori* in patients given NSAIDs (1, 4, 23). In vivo experimental studies have shown that NSAIDs revert the increase in apoptosis and proliferation of gastric epithelial cells as well as the inflammatory activity caused by *H. pylori* (22, 60). The interference of NSAIDs with other *H. pylori*-related effects in the gastric mucosa is still unknown. In particular, whether and how NSAIDs affect VacA-induced cytotoxicity has not been determined.

This study was designed to evaluate the relationship between NSAIDs and *H. pylori* VacA toxin in terms of vacuolation of human gastric epithelial cells in culture. Our data show that 1) NSAIDs inhibit VacA-induced cell vacuolation independently of inhibition of cell proliferation and PG synthesis; 2) NSAIDs mainly impair vacuole development/maintenance without affecting cell binding and internalization of VacA; and 3) NSAIDs, as well as the chloride channel blocker 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB), also inhibit cell vacuolation induced by ammonia.

**MATERIALS AND METHODS**

Human gastric epithelial cells in culture. We used the MKN 28 cell line. This cell line derives from a human gastric tubular adenocarcinoma and shows gastric-type differentiation (18, 45). MKN 28 cells were grown as monolayers in DMEM-Ham’s nutrient mixture F-12 (1:1; Sigma, St. Louis, MO) supplemented with 10% FCS (GIBCO-BRL, Paisley, UK) at 37°C in a humidified atmosphere of 5% CO₂ in air.

Bacterial broth culture filtrate preparation. VacA-containing broth culture filtrate (VacA⁺ BCF) was produced from *H. pylori* strain 60190 (ATCC 49503), as described by Ricci et al. (39, 42). Briefly, bacteria were grown in Brucella broth (Difco, Detroit, MI) supplemented with 1% Vitox (Oxoid, Basingstoke, UK) and 5% FCS (GIBCO) for 24–36 h at 37°C under microaerobic conditions and continuous shaking. When bacterial suspensions reached 1.2 optical density units at 450 nm (corresponding to a bacterial concentration of 5 × 10⁸ colony-forming units/ml), bacteria were removed by centrifugation (12,000 g for 10 min), and the supernatant was sterilized by passage through a 0.22-μm cellulose acetate filter. BCF was then concentrated 50-fold by using centrifugal filter devices (Millipore, Bedford, MA). Concentrated VacA⁺ BCF was stored at −20°C and used for cell intoxication at a dilution of 1:150. VacA prepared by this procedure does not require activation by acid/alkaline treatment (40). The vacuolating power of VacA⁺ BCF prepared and used as described was equivalent to that exhibited by a final concentration of 0.4 μg/ml purified VacA.

NSAIDs. We used 1) indomethacin [1-(p-chlorobenzoyl)-5-methoxy-2-methyl-3-indolylacetic acid (Indo; Sigma); and 2) aspirin [2-(acetylsalicylic acid (ASA); Sigma); and 3) N-(2-cyclohexyloxycarbonyl)-5-nitrobenzenesulfonamide (NS-398; Calbiochem, La Jolla, CA).

**Cell vacuolation.** Subconfluent monolayers of MKN 28 cells on six-well multwell tissue culture dishes were incubated for 30 min at 37°C with Hanks’ balanced salt solution (HBSS) in the absence or presence of different NSAIDs, PGE₂ or arachidonic acid (AA). Then either 4 mM NH₄Cl, 4 mM NH₄Cl plus VacA⁺ BCF, or NH₄Cl at different concentrations in the absence or presence of VacA⁺ BCF was added for 16 h. In some experiments, to study the effect of NSAIDs on an already developed VacA⁺-induced cell vacuolation, cells were incubated for 16 h with 4 mM NH₄Cl or 4 mM NH₄Cl plus VacA⁺ BCF and then for an additional 8 h in the absence or presence of different NSAIDs (VacA⁺-preloaded protocol). At the end of each experiment, cell vacuolation was quantitated by means of neutral red uptake, carried out in accordance with Cover et al. (7). Results were expressed as micrograms of neutral red per microgram cell protein (41).

The protein content of cell monolayers was measured in accordance with Lowry et al. (28). Neutral red is an acidophilic dye that binds specifically to the acidic compartments of the cell and can be used to evaluate cell vacuolation (42). Vacuolation of variously treated cell monolayers was also evaluated qualitatively using a phase-contrast inverted microscope (Diaphot 300; Nikon, Tokyo, Japan) equipped with a photocamera; representative microscopic fields were photographed by a technical assistant (V. Necchi) unaware of the treatment.

**Cell proliferation.** Cell proliferation was assayed as previously described (39). Briefly, MKN 28 cells were seeded on 24-well multwell tissue culture dishes (2 × 10⁴ cells/well). After seeding (12 h), the FCS-containing medium was removed and replaced with FCS-free medium to synchronize cell cycles. Later (12 h), cells were incubated for 24 h in the absence or presence of either different NSAIDs or 1-β-D-arabinofuranosylcytosine (ARA-C) in medium with dialyzed FCS. Before the end of the incubation (4 h), [3H]thymidine (1.8 μCi/well; Amersham International, Little Chalfont, UK) was added. At the conclusion of the incubation period, cells were washed three times with ice-cold PBS, 10% TCA was added, and the precipitate was passed through glass microfiber filters (Whatman 934 AH) and washed with 100% ethanol. Filters then were transferred to vials containing 10 ml of scintillation cocktail (Econofluor; DuPont de Nemours Italiana, NEN Products, Cologno Monzese, Italy) and counted in a Beckman beta counter. The results were expressed as a percentage of [3H]thymidine uptake in control samples (i.e., samples not treated with NSAIDs or ARA-C).

**VacA binding and internalization.** Subconfluent monolayers of MKN 28 cells were incubated with HBSS in the absence or presence of different NSAIDs for 30 min at 37°C and then cells were transferred at 4°C for 1 h to block the active cellular processes required for VacA internalization (30). VacA⁺ BCF was added, and cells were incubated for 30 min at 4°C. At the end of this incubation period, the medium was discarded, and the monolayers were washed three times with ice-cold HBSS. For VacA binding evaluation, cells were then lysed with boiling lysis buffer (1.5 M Tris-HCl, pH 6.8, 8% SDS, and 40% glycerol; supplemented with 20% 2-mercaptoethanol) and collected. For VacA internalization evaluation, cells were then incubated for 4 h at 37°C with HBSS containing 4 mM NH₄Cl in the absence or presence of different NSAIDs. As described by McClain et al. (30), increasing the temperature from 4 to 37°C allows bound VacA to become internalized in cells. After 37°C incubation, the medium was
discarded, and cells were incubated for 1 h at 4°C with HBSS containing 2 mg/ml Pronase (Calbiochem) to proteolyze extracellular membrane-bound VacA. At the end of this incubation, cells were completely detached from the plastic support by repeated pipetting and centrifuged at 300 g for 2 min at 0°C. Cells were then lysed with boiling lysis buffer and collected. VacA binding and internalization were evaluated by SDS-PAGE followed by Western blotting as previously described (48) using a polyclonal rabbit anti-VacA serum (serum 958; kindly provided by T. L. Cover, Nashville, TN) and the enhanced chemiluminescence (ECL) revelation system (Amersham). VacA immunoreactive band intensity was quantitated by densitometric scanning of ECL-exposed films using a Howtek Scanmaster-3 densitometer with RFL Print software (Pharmacia Biotech, Cologno Monzese, Italy). VacA binding and internalization studies were normalized internally in accordance with McClain et al. (30); for any given experiment, equal amounts of VacA were added to equal numbers of cells, the cells were lysed in equal volumes, and equal volumes of cell lysate were separated by SDS-PAGE.

**Assay of cytotoxicity of diphtheria and ricin toxins.** Measurement of cell protein synthesis inhibition by diphtheria toxin (DT) and ricin toxin (RT) was carried out essentially as described by Moya et al. (31). Briefly, subconfluent monolayers of MKN 28 cells on 24-well multiwell tissue culture dishes were washed two times with HBSS and then 0.5 ml of DMEM (GIBCO) containing 2% FCS was added to each well. Cells were preincubated for 30 min at 37°C in the absence or presence of ASA or NS-398, and then DT or RT at the final concentrations of 10⁻⁹ or 10⁻¹⁰ M were added, and the cells were incubated at 37°C. After 16 h of incubation, the medium was removed, and, after washing, 0.5 ml of an FCS- and leucine-free medium that contained 0.25 μCi [¹⁴C]leucine (Amersham) was added in each well for 2 h. This medium was then removed, and 0.5 ml of 10% TCA was added to each well. After 2 h at 4°C, the precipitated monolayers were washed three times with 10% TCA, and 0.5 ml of 1 M NaOH was added to each well to dissolve the cell monolayer. Solubilized monolayers were transferred to counting vials containing the scintillation cocktail, and the radioactivity was counted in a beta counter. The results were expressed as a percentage of [¹⁴C]leucine incorporation in control samples (i.e., samples not treated with toxins).

**Statistics.** Results were expressed as means ± SE of three independent experiments. The statistical significance of the differences was evaluated by ANOVA followed by Newman-Keuls’ Q-test. Data expressed as a percentage of control were analyzed before being normalized vs. control.

**RESULTS**

**NSAIDs** both prevented and reverted VacA-induced cell vacuolation. Figure 1 shows that either Indo or ASA, nonselective inhibitors of cyclooxygenase (COX) isoforms, added 30 min before VacA (i.e., VacA afterloaded protocol), dose-dependently and significantly (P < 0.05) inhibited VacA-induced vacuolation of MKN 28 cells, causing an ∼55–70% reduction in neutral red uptake with the highest dose of NSAIDs. A lower dose of Indo (i.e., 0.01 mM) did not cause any significant inhibition of neutral red uptake (data not shown). We also tested whether selective inhibition of COX-2, the inducible isoform of the enzyme responsible for PG production, exerted similar effects and found that NS-398 (a highly selective inhibitor of COX-2) caused an inhibition of VacA-dependent cell vacuolation compa-
rable to that obtained with nonselective COX inhibitors (Fig. 1).

We asked whether NSAIDs exerted any effect also on already developed VacA-induced cell vacuoles by treating MKN 28 cell monolayers previously incubated with VacA⁺ BCF and 4 mM NH₄Cl (i.e., VacA preloaded protocol) with NSAIDs. We found that all NSAIDs used in the present study caused a statistically significant (P < 0.05) dose-dependent reversion of VacA-induced cell vacuolation (Fig. 1).

To be sure that the effects we found were actually on cell vacuolation and not just on neutral red uptake, we qualitatively evaluated the effect of NSAIDs on VacA-induced cell vacuolation by means of phase-contrast microscopy. Figure 2 shows that incubation with VacA⁺ BCF in HBSS containing 4 mM NH₄Cl caused a massive cell vacuolation (more evident at the periphery of cell islets) compared with control, VacA-untreated cells (Fig. 2, B vs. A). ASA, in a concentration that did not cause any morphological change to the cells (Fig. 2C), caused a dramatic reduction in VacA-dependent cell vacuole formation (Fig. 2D). A comparable qualitative effect was observed with both Indo and NS-398 (data not shown).

Fig. 2. Phase-contrast microphotographs of subconfluent MKN 28 cell monolayers showing the effect of ASA on VacA-dependent cell vacuolation. A: HBSS containing 4 mM NH₄Cl; B: HBSS containing 4 mM NH₄Cl in the presence of VacA⁺ BCF; C: HBSS containing 4 mM NH₄Cl in the presence of 1 mM ASA; D: HBSS containing 4 mM NH₄Cl in the presence of VacA⁺ BCF and 1 mM ASA. MKN 28 cells were incubated for 16 h; 1 mM ASA was added 30 min before the addition of NH₄Cl and VacA⁺ BCF. Original magnification: ×100.

**Fig. 3.** Effect of PGE₂ or arachidonic acid (AA) on the action of different NSAIDs on VacA-induced vacuolation of MKN 28 cells. Neutral red dye uptake was measured after 16 h of cell incubation with either HBSS containing 4 mM NH₄Cl (control) or VacA⁺ BCF in HBSS containing 4 mM NH₄Cl, in the absence or presence of either Indo, ASA, or NS-398 added 30 min before the addition of VacA⁺ BCF. Both PGE₂ and AA were added immediately before incubation with NSAIDs. Data are means ± SE of 3 independent experiments. *P < 0.05 vs. paired control. °P < 0.05 vs. paired VacA⁺ BCF in the absence of NSAIDs.

**NSAID-dependent inhibition of VacA vacuolating activity is not accounted for by either inhibition of PG synthesis or inhibition of cell proliferation.** NSAIDs inhibit PG generation by blocking COX activity (15, 55). In particular, at the doses used in the present study, Indo, ASA, or NS-398 inhibit PGE₂ production by MKN 28 cells by >70% (Ref. 45 and unpublished observations). To assess whether the NSAID effect on VacA vacuolating activity might be the result of inhibition of PG synthesis, we analyzed whether exogenously added PGE₂ or the PG precursor AA counteracted NSAID-mediated inhibition of VacA-induced cell vacuolation of MKN 28 cells. As shown in Fig. 3, neither PGE₂ nor AA affected NSAID-dependent inhibition of VacA-induced cell vacuolation.

The fact that subconfluent cell monolayers (i.e., actively proliferating cells) are much more sensitive to VacA vacuolation than confluent monolayers (37) rises the possibility that the vacuolating inhibitory effect of NSAIDs might be the result of inhibition of cell proliferation. Because NSAIDs at the concentrations used in this study were able to cause a statistically significant (P < 0.05) inhibition of [³H]thymidine uptake by MKN 28 cells in a dose-dependent fashion (Fig. 4), we tested the effect of different doses of ARA-C, an NSAID-unrelated DNA synthesis inhibitor, on either [³H]thymidine uptake or VacA-induced neutral red uptake by subconfluent MKN 28 cell monolayers. Figure 5 shows that ARA-C exerted no effect on VacA-induced cell vacuolation while causing a statistically significant...
treated cells (lanes b, c, and d). Densitometric analysis of ECL-exposed films confirmed the absence of significant difference in VacA immunoreactive band intensity between control untreated and NSAID-treated cells (data not shown). In the binding experiments, the absence of VacA immunoreactivity in protease-treated cells compared with protease-untreated cells (Fig. 6A, lane e vs. lane a, respectively) confirmed that all cell-associated VacA was really extracellularly bound. In the internalization experiments, a VacA immunoreactive band of slightly higher intensity was observed in protease-untreated compared with protease-treated cells (Fig. 6B, lane e vs. lane a, respectively), suggesting, in agreement with previous findings (30), that not all bound VacA was internalized.

To further investigate whether the effect of NSAIDs was specific for VacA-induced cytotoxicity (i.e., cytoplasmic vacuolation) or, on the contrary, a more general phenomenon operating also against the cytotoxic action of other protein toxins with an intracellular target, we studied NSAID effects on the action of DT and RT. In this respect, we evaluated the effects of ASA and NS-398 on protein synthesis inhibition induced by either DT or RT in MKN 28 cells. As shown in Fig. 7, neither DT nor RT cytotoxicity was significantly altered by the NSAIDs we used.

NSAIDs or the chloride channel blocker NPPB similarly prevented both ammonia- and VacA-dependent cell vacuolation. Altogether the above results suggested that NSAIDs counteracted VacA vacuolating activity by inhibiting vacuole development/maintenance through a PG production-independent, yet unidentified, mechanism. Moreover, our results showed that NSAIDs exerted no protection against other protein toxins with intracellular targets. Weak bases, such as ammonia, are known to cause the formation of cell vacuoles, which are much smaller than, but qualita-
study to assess whether NSAIDs affected cell vacuolation induced by *H. pylori* VacA cytotoxin in an experimental model where the effects of systemic factors, gastric acid secretion, or inflammatory infiltrate are negligible.

We demonstrated that both Indo and ASA, nonselective inhibitors of COX activity, are able not only to significantly inhibit VacA-induced vacuole formation but also to cause a significant reversion of vacuoles already developed in VacA-intoxicated cells. That NS-398, a highly selective inhibitor of COX-2 isoform, is as effective as Indo and ASA in counteracting VacA vacuolating action may suggest that inhibition of COX-2, more than COX-1, might contribute to NSAID-dependent inhibition of VacA cytotoxicity. However, even though NS-398 is 42-fold more selective for COX-2 than it is for COX-1 (29), based on our results, we cannot completely rule out a role for COX-1 inhibition in the antivacuolating effect of NSAIDs.

The concentrations of NSAIDs used in this study are compatible with in vivo therapeutic doses of the drugs. In fact, one 50-mg tablet of Indo or one 500-mg tablet of ASA in ~50 ml of water give a final instilled concentration of 2.8 and 55 mM, respectively. Moreover, the concentrations of NSAIDs used in vivo in experimental animals range in different studies from 1.7 to 17 mM for Indo (33, 54), from 11 to 130 mM for ASA (17, 56), and from 1.9 to 19 mM for NS-398 (24, 54). The in vivo relevance of our in vitro study depends on the ability of NSAIDs to permeate the mucus layer lining the mucosal surface of the stomach. In this regard, the mucus gel is readily permeated by exogenous damaging agents such as ethanol, NSAIDs, or bile salts (2, 12). Moreover, ASA and other NSAIDs, at the intragastric

DISCUSSION

NSAIDs and *H. pylori* are the major determinants of gastroduodenal ulcerations in humans (15, 26). Whether *H. pylori* infection exerts beneficial or detrimental effects on NSAID-related gastric toxicity is still controversial (1, 4, 16, 21, 23, 25). We designed this
acidic pH, are largely undissociated (i.e., lipid soluble), being able to permeate the apical cell membrane of surface epithelial cells where they exert their topical effects (12, 20). However, NSAIDs also act on the stomach systemically, following absorption and subsequent delivery to the basolateral membrane of gastric epithelial cells through the gastric microcirculation. Therefore, the results of the present study, conducted in an experimental model where systemic factors such as blood flow are excluded, must be interpreted with caution, also taking into account mucus permeation by drugs.

The main effects of NSAIDs on gastric mucosa are accounted for by inhibition of PG generation through the blockade of COX activity (15, 55, 57). We therefore assessed whether NSAID-dependent inhibition of VacA vacuolating activity might be related to the inhibition of PG synthesis. Our data suggest that the effect of NSAIDs on VacA-induced cell vacuolation was independent of inhibition of PG production because it was completely insensitive to addition of either PGE2 or AA. However, we cannot rule out that additional PGs other than PGE2 may be contributing to these events. That exogenously added PGs did not counteract NSAID action suggests that the effect of NSAID on VacA vacuolation might be contributed to by COX-unrelated mechanisms (58).

VacA-induced vacuole formation in epithelial cells largely depends on the degree of confluency of the cell monolayer, with confluent monolayers exhibiting a highly reduced cell vacuolation compared with subconfluent ones (reviewed in Ref. 37). In addition, in our experience with subconfluent MKN 28 cell monolayers and low doses of VacA, cells at the periphery of cell islets always show vacuoles both much earlier and larger compared with cells occupying inner positions (Fig. 2 and unpublished observations). The hypothesis thus arises that highly proliferating cells are more sensitive to the vacuolating effect of VacA compared with quiescent cells and that NSAID-dependent inhibition of VacA cytotoxicity might be secondary to NSAID antiproliferative action. That the NSAID-unrelated DNA synthesis inhibitor ARA-C, at doses causing an inhibition of cell proliferation very similar to that we observed with NSAIDs, exerted no effect on VacA-induced cell vacuolation of MKN 28 cells rules out this possibility.

Mounting evidence suggests that VacA vacuolating activity requires toxin binding and internalization by eukaryotic cells (reviewed in Ref. 37). Recently, it has been suggested that protein toxin endocytosis may be regulated by the COX pathway (27). We therefore asked whether the NSAID antivacuolating effect might be due to an impairment of the process of cell intox-
cation by VacA. However, we showed that NSAIDs did not affect VacA binding to the cell plasma membrane nor did they alter VacA internalization efficiency by MKN 28 cells.

To address the question whether NSAIDs were able to protect MKN 28 cells not only against VacA but also against other protein toxins with intracellular targets, we studied the effect of NSAID on DT- and RT-dependent cytotoxicity. These toxins are internalized in the cells, reach their cytoplasmic targets, and inhibit protein synthesis through different mechanisms (reviewed in Refs. 5 and 34). Our data demonstrated that subconfluent MKN 28 cell monolayers were sensitive to flurbiprofen and on VacA-dependent potentiation of ammonia-induced vacuolation. Our results might be accounted for by the different experimental models. In partial support of this hypothesis, we found that, in MKN 28 gastric cells, 0.01 mM NPPB gave a significant inhibition of VacA-induced vacuole formation, whereas a 10-fold higher concentration (i.e., 0.1 mM NPPB) was necessary to obtain comparable effects in HeLa cells (52).

In conclusion, our data show that Indo, ASA, and NS-398 protected MKN 28 cells against VacA-induced cytotoxicity via a mechanism independent of PG production, inhibition of cell proliferation, and cell binding/internalization of VacA. We postulate that NSAIDs may act by inhibiting VacA channel activity and endogenous anionic channels required for vacuole genesis/maintenance. Because VacA is a major determinant of gastric colonization by H. pylori (44, 47, 51) and of H. pylori-related gastroduodenal injury, our data showing a protective effect of NSAIDs against VacA cytotoxicity are in partial support of the theory that NSAIDs and H. pylori may not act synergistically in damaging the gastric mucosa.

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