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Commentary

Where is the evidence that cyclooxygenase inhibition is the primary cause of nonsteroidal anti-inflammatory drug (NSAID)-induced gastrointestinal injury? Topical injury revisited

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Abstract

In this commentary, we take a critical look at the concept that the gastrointestinal (GI) side-effects of nonsteroidal anti-inflammatory drugs (NSAIDs) are due to the ability of these drugs to inhibit cyclooxygenase-1 (COX-1) that is constitutively expressed in the GI mucosa. Indeed, development of the new "super aspirins," such as *Celebrex* and *Vioxx*, that selectively inhibit the inducible COX-2, expressed in areas of inflammation, is a direct outgrowth of this concept. We discuss evidence from both the laboratory and the clinic that appears to be inconsistent with the above concept, and cite a number of examples where the depletion of mucosal prostaglandin levels and the development of GI injury can be dissociated. Instead, we revisit the possibility that NSAID-induced GI side-effects are mostly due to the ability of these drugs to topically injure the GI mucosa. We devote the remainder of the commentary to presenting evidence from our and other laboratories that NSAIDs can directly attenuate the surface hydrophobic barrier of the GI mucosa due to their ability to bind to zwitterionic phospholipids, and that even systemically administered NSAIDs that are secreted into the bile may induce GI ulceration and/or bleeding due to phospholipid interactions and the development of topical mucosal injury.

Keywords: Cyclooxygenase (COX); Nonsteroidal anti-inflammatory drugs (NSAIDs); Aspirin; Prostaglandins; Surface hydrophobicity; Surfactant phospholipids; Phosphatidylcholine

1. Introduction

Ever since its discovery, 100 years ago by Felix Hoffman of the Bayer Corporation, aspirin has remained the most heavily consumed "over the counter" drug, with 20–30 billion tablets purchased annually in just the United States alone [1–4]. It has been estimated that 1–2% of the world population consumes at least one aspirin tablet per day. The popularity of aspirin has been predicted to increase even further in the future as evidence has become available that the drug is effective not only in inhibiting fever, pain, and inflammation, but also in reducing the risk of developing heart disease, stroke, thrombosis, colon cancer, and Alzheimer's disease [4–7]. It is also well established that aspirin is a member of the NSAID family that share the ability of inhibiting fever, pain, and inflammation by blocking the formation of prostaglandins by either reversibly or irreversibly (in the case of aspirin) inhibiting the rate-limiting enzyme COX [1,7–10]. There are at least fifteen NSAIDs on the market at present and, because of their increased potency to inhibit COX activity, they are frequently prescribed (~70 million NSAID prescriptions are written per year) for individuals suffering from chronic inflammation and pain, such as the 10–15 million Americans inflicted with osteo- or rheumatoid arthritis. One of the major problems with this trend is that NSAID usage is associated with a number of side-effects, mostly affecting the upper GI tract and the kidney. The most common and disturbing of these side-effects are NSAID-induced gastroduodenal ulceration and bleeding, which have been reported to be present in 15–30% of chronic NSAID users [11–14]. GI hemorrhage and perforation, associated with the usage of aspirin and other NSAIDs, appear to be increasing annually and presently are responsible for ~$5–10 billion dollars in hospitalization

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Abbreviations: NSAID, nonsteroidal anti-inflammatory drug; COX, cyclooxygenase; GI, gastrointestinal; and PC, phosphatidylcholine.

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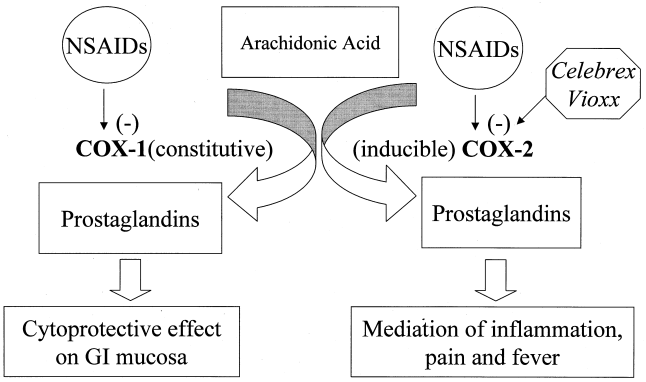

Fig. 1. Schematic depiction of the concept that NSAID-induced GI injury can be prevented by sparing the COX-1 constitutive enzyme of the mucosa, by the use of COX-2 selective inhibitors that specifically block the inducible COX-2 isoform expressed at sites of inflammation.

charges and lost work time, and 25,000 deaths per year. Interestingly, a number of laboratories have reported that ~30–60% of our population have a GI intolerance for aspirin and the more potent NSAIDs [15,16], and that simply reducing the dose of aspirin does not prevent the development of gastroduodenal erosions and ulcerations in these susceptible individuals [17,18].

Because of these problems and the large market potential, pharmaceutical houses have made a sustained effort to develop a GI-safe NSAID. The greatest effort in recent times has been put forth to develop a family of NSAIDs that selectively inhibit the type-2 isoform of the COX enzyme. The COX-2 protein, which has approximately the same molecular weight as COX-1 (65–70 kDa), is encoded by a related but different gene [19–23]. Unlike the COX-1 gene, which is constitutively expressed in tissues like the GI mucosa and kidney, COX-2 is not normally expressed in most tissues, being induced during periods of inflammation. This led to the thinking that the development of NSAIDs that are selective inhibitors of COX-2 would target sites of inflammation (e.g. joints), leaving the constitutively expressed "cytoprotective" prostaglandins in the stomach unaffected (see Fig. 1 for a schematic depiction of this concept). This possibility was supported by laboratory studies, which have reported that a number of COX-2-selective NSAIDs have low gastric toxicity in animal models of ulcer disease [20,24], and most recently by encouraging results of Phase II and III clinical studies by clinical investigators in collaboration with scientists at Monsanto-Searle and Merck-Dupont, in their evaluation of their respective COX-2 inhibitors, Celecoxib (sold under the name *Celebrex*) and Rofecoxib (sold under the name *Vioxx*) [24–28]. Recent clinical studies, which report that there are significantly fewer gastroduodenal erosions and ulcers in human subjects acutely and chronically taking a selective COX-2 blocker than a "therapeutically equivalent dose" of a conventional NSAID (e.g. ibuprofen, diclofenac) [28], and a very aggressive marketing campaign have contributed to the enormous success (based on sales reports) of both *Celebrex* and *Vioxx*, shortly after the launching of these drugs.

Although the evidence presently indicates that both Celecoxib and Rofecoxib have low gastroduodenal toxicity profiles in humans in comparison to conventional NSAIDs, questions remain concerning the role of COX-1 inhibition in the pathogenesis of NSAID-induced gastropathy, and the potential importance of COX-2 in mucosal integrity and healing. Let us consider the evidence that supports and/or refutes the hypothesis that NSAIDs induce GI injury by inhibiting COX-1, thereby depleting the mucosal tissue of "cytoprotective" prostaglandins. Clearly, a number of highly regarded and cited papers support the concept that NSAIDs primarily induce GI injury by inhibiting COX activity of the gastric mucosa (which has been demonstrated to be predominantly COX-1) [29–35]. These studies demonstrated, in both animals and humans, that NSAID-induced gastroduodenal erosions, ulcerations, and bleeding are frequently associated with a decrease in mucosal prostaglandin concentration and/or COX activity, and that the exogenous administration of prostaglandin analogues (e.g. Misoprostil) with "cytoprotective" activity can either partially or completely reverse the injurious effects of NSAIDs on the GI mucosa. However, these findings cannot be construed as definitive proof of the above theory, since there are multiple reports in the literature that prostaglandin administration generically protects the GI mucosa against numerous damaging agents and/or conditions that (unlike NSAIDs) do not cause a depletion in tissue levels of prostaglandins [29,30].

More importantly, the above hypothesis is not supported by a rather large body of evidence that indicates that the linkage between COX inhibition and GI injury/bleeding is not very strong. For example, Ligumsky and associates published a series of papers on studies in rats and dogs that appeared to dissociate COX inhibition from mucosal injury [36–38]. Initially, they demonstrated that aspirin and its metabolite, salicylic acid, had equivalent abilities to induce injury to the canine gastric mucosa, mounted in Ussing chambers, even though aspirin depleted the tissue of "cytoprotective" prostaglandins, whereas salicylic acid displayed no COX inhibitory activity [36]. In subsequent rodent studies, it was demonstrated that mucosal COX activity was inhibited by >90% regardless of route of administration, subcutaneously or intragastrically, of the aspirin, although ulcerations only formed in the stomachs of rats when the NSAID was administered intragastrically [37] (see Fig. 2). Whittle [39] also reported a dissociation between the effect of indomethacin to induce COX inhibition and mucosal injury in the small intestine, as intestinal lesions only began to develop 48 hr after NSAID administration, at a time point when COX activity (which was fully inhibited <3 hr, post-indomethacin) had returned to normal. These workers, however, felt that they did have evidence for a linkage between indomethacin-induced gastric injury (which occurred hours after treatment) and COX inhibition, although our laboratory has obtained evidence recently that these properties

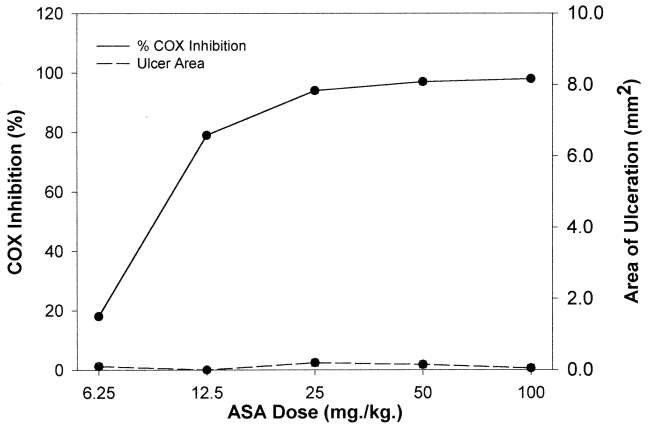

Fig. 2. Redrawing of the results of Ligumsky *et al.* [37] that demonstrate that the parenteral administration of aspirin (ASA) induces a dose-dependent decrease in gastric mucosal COX activity (inducing a >95% depletion of gastric prostaglandin concentration) without causing gastric ulceration in rats. In contrast, intragastric administration of aspirin induced significant gastric injury in rats.

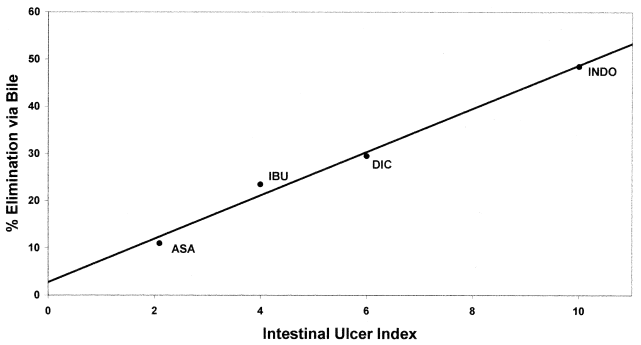

Fig. 3. Redrawing of the results of Brune *et al.* [43] that demonstrate the striking relationship between the ability of an NSAID to be secreted into the bile and the induction of intestinal ulceration in rats. Abbreviations: ASA, aspirin; IBU, ibuprofen; DIC, diclofenac; and INDO, indomethacin.

were not significantly associated, even during this acute stage [40].

A number of laboratory studies have compared the COX inhibitory efficacy and the GI toxicity of several non-aspirin NSAIDs when given by different routes of administration (intragastric vs rectal or parenteral) [37,38]. These studies demonstrated that the GI damaging effect of certain NSAIDs (sulindac, ibuprofen, and aspirin) was dependent on the intragastric delivery of these drugs, although they all induced maximal mucosal COX inhibition, regardless of the route of administration. An interesting common characteristic of the NSAIDs that induce GI mucosal injury when administered systemically (indomethacin, diclofenac, or ketoprofen) is that they are all secreted into the bile and enter the enterohepatic transport system [41–43]. Brune *et al.* [43] have compiled data in rats demonstrating that a highly significant association exists between the percentage of the administered dose of an NSAID that is secreted into the bile and the ability of the NSAID to induce GI ulceration (see Fig. 3). The possibility that these drugs may induce topical injury to the mucosa by being secreted into the bile is further supported by evidence from a number of laboratories that mucosal injury due to the systemic administration of these NSAIDs can be prevented by bile duct ligation [41, 42]. It is certainly worth noting that the few animal studies that have demonstrated that systemic aspirin administration induces gastric injury, purportedly by gastric COX inhibition, have been performed exclusively on cats [31,44,45]. Interestingly, it is well known among veterinarians that aspirin is extremely toxic and not recommended for cats, most likely due to the excessively long metabolic half-life of the drug (with the $T_{1/2}$ of aspirin and its metabolite, salicylic acid, being 10–100 times longer in cats than in most species), which may be attributable to the inability of cats to glucuronidate drugs [46–50].

It should be pointed out that the evidence suggesting that mucosal COX inhibition may not be directly involved in the pathogenesis of NSAID-induced enteropathy is also supported by a number of clinical studies. Several studies have reported that i.v. administration of aspirin does not cause detectable histological injury to the human gastric mucosa, in contrast to oral administration of the NSAID at the same dosage [51,52]. It was also reported that after 2–4 weeks of NSAID treatment, the human gastric mucosa becomes resistant to the injurious actions of oral aspirin or indomethacin, and that this adaptive response was not linked to a recovery of COX activity, which remained fully blocked during the study period [53,54]. In a recently published study, Cryer and Feldman [55] compared the ability of aspirin orally administered to healthy volunteers at 10, 81, and 325 mg/day for 10 days to induce gastroduodenal and rectal lesions and mucosal prostaglandin depletion. Although the major point of the study was to demonstrate that aspirin even at the lowest dose was damaging to the gastroduodenal mucosa, it became clear that the dose-dependence of endoscopic injury and mucosal prostaglandin levels were not linked. This led the investigators to state "when we assessed for a potential correlation between mucosal injury scores and mucosal prostaglandin content in our group of 29 subjects, in no mucosal region was a significant correlation observed. Furthermore, subjects who developed ulcers were not necessarily those with the lowest mucosal prostaglandin levels from baseline after aspirin treatment." The authors then concluded that their "observations suggest that whereas the lowering of GI mucosal prostaglandin levels is probably a prerequisite for GI mucosal injury by aspirin, it is not sufficient as an exclusive factor."

The hypothesis that NSAIDs induce GI injury, primarily by inhibiting mucosal COX-1, predicts that a COX-1 selective inhibitor should be very toxic to the GI mucosa and that mice deficient in the isozyme, due to targeted gene disruption, would be prone to the development of spontaneous mucosal ulcers and be more sensitive to NSAIDs than their wild-type littermates. However, neither possibility has proven to be the case, as it has been reported that a newly developed COX-1-selective NSAID (SC-560) induces no

GI injury in rats, even when administered at a high dose, whereas it becomes GI toxic when given together with a selective COX-2 blocker [56]. Furthermore, Langenbach *et al.* [57] reported that COX-1 null animals had no detectable GI ulcer disease and, if anything, were more resistant to indomethacin-induced ulcer development. To make matters more confusing, Morham *et al.* [58] found in a subsequent study that COX-2 knockout mice were not viable and frequently succumbed to peritonitis and renal disease. The possibility that COX-2 inhibition may be detrimental has also been supported by a number of animal studies that indicate that the healing of ulcers in the proximal and distal gut is exacerbated if animals are treated with selective COX-2 blockers [59–62]. Recent preclinical studies by members of Peskar's laboratory have also indicated that selective COX-2 inhibitors exacerbate ischemia/reperfusion injury to the gut and block the ability of the mucosa to mount an "adaptive cytoprotective" response, where exposure to a mild irritant protects against a subsequent ulcerogenic challenge [61]. Similar complications of COX-2 selective inhibitors in ulcer formation and/or healing in humans have not been reported to date, although recent evidence has emerged that these drugs have limited efficacy in the reduction of dyspeptic symptoms. Lastly, it should be pointed out that even if all the claims of the superior safety at comparable therapeutic doses of COX-2 selective inhibitors are ultimately proven, by definition COX-2 inhibitors should have little or no utility for the millions of individuals that are consuming low-dose aspirin for cardiovascular risk reduction (due to the ability of the drug to inhibit the COX-1 activity of platelets). This fact is placed in perspective by one clinical investigator who has reported that low-dose aspirin users represent the largest group of patients admitted to his hospital due to GI hemorrhage [63].

Based on the evidence documented above, a compelling case can be made to investigate other mechanisms by which NSAIDs may induce GI mucosal injury, and how this information can be used to develop alternative strategies to reduce or prevent the GI toxicity of these compounds. Other potential targets of NSAID-induced enteropathy are the ability of these drugs to: reduce mucosal blood flow and induce leukocyte adherence to the vascular wall, which led to the development of NO-NSAIDs [64]; uncouple mitochondrial oxidative phosphorylation [65]; induce cellular acidification due to their protonophore characteristics [66]; and attenuate the hydrophobic, non-wettable characteristics of the mucosa, thereby increasing the susceptibility of the tissue to luminal acid [67]. Since the interaction of NSAIDs with the majority of the above GI properties has been the subject of review articles by experts in the respective field of study, I will focus the remainder of this commentary on the effects of NSAIDs on the most apical barrier, the surface hydrophobic and phospholipidic lining of the GI mucosa, an area our laboratory has been studying intensively over the past 10–15 years.

This approach was an outgrowth of observations in our laboratory that the surface of the gastric mucosa of a number of laboratory animals possessed hydrophobic properties, as determined by contact angle analysis [68–70]. Subsequent clinical endoscopic studies demonstrated that the human gastric mucosa similarly possessed hydrophobic surface properties [71]. We argued that, similar to hydrophobic industrial surfaces that are protected from environmental corrosion, such a hydrophobic lining would make the epithelial surface non-wettable to luminal acid. Biochemical and ultrastructural evidence strongly suggested that this property was attributable to the ability of gastric surface mucous cells to synthesize and secrete a surfactant-like phospholipid, which accumulated within, and coated, the mucus gel layer [72–74]. Since phosphatidylcholine (PC) represented the most abundant and surface-active of the gastric phospholipids, we initiated a series of rodent experiments to demonstrate that PC could protect rats from a number of ulcerogenic agents and/or conditions including NSAIDs [69,75]. At the same time, Goddard and coworkers from our laboratory demonstrated that aspirin exposure induces a rapid and dose-dependent decrease in the surface hydrophobicity of the canine gastric mucosa, mounted in Ussing chambers [73,74]. A similar response was seen by other NSAIDs under both *in vitro* and *in vivo* conditions [68,75,76]. Our understanding of the mechanism by which aspirin and other NSAIDs reduce the surface hydrophobicity of the gastric mucosa was increased with evidence that NSAIDs, as a class, have a strong ability to chemically associate with PC under both organic and aqueous conditions [75]. Furthermore, Giraud *et al.* [76] employed a number of fluorescent hydrophobic probes to confirm that non-aspirin, NSAIDs (indomethacin and naproxen) chemically interact with PC liposomes, resulting in significant alterations in both their hydrophobicity and fluidity. This led us to speculate that NSAIDs chemically react with and destabilize the intrinsic phospholipid lining of the mucus gel layer, resulting in an attenuation in the hydrophobic barrier of the stomach to luminal acid. A schematic model of how topically applied NSAIDs can interact with and destabilize an extracellular phospholipid lining is depicted in Fig. 4. The model proposes that the available anionic group of aspirin and other NSAIDs (which are undissociated at acidic pH values of gastric juice) will chemically ionize and associate with the positively charged head group of PC as the drug begins to enter and is exposed to the pH gradient within the mucus gel layer [75]. This chemical interaction between PC and the NSAID will result in a change in the physico-chemical properties of both reactants and a dissolution of the phospholipidic hydrophobic lining [75,76]. Based upon this hypothesis, we reasoned that by chemically pre-associating PC to aspirin and other NSAIDs we could prevent this interaction from occurring and maintain the hydrophobic barrier properties of the stomach. Our laboratory studies confirmed this possibility as we demonstrated that the gastric toxicity of aspirin-PC and other PC-associated NSAIDs was markedly lower than the unmodified drug

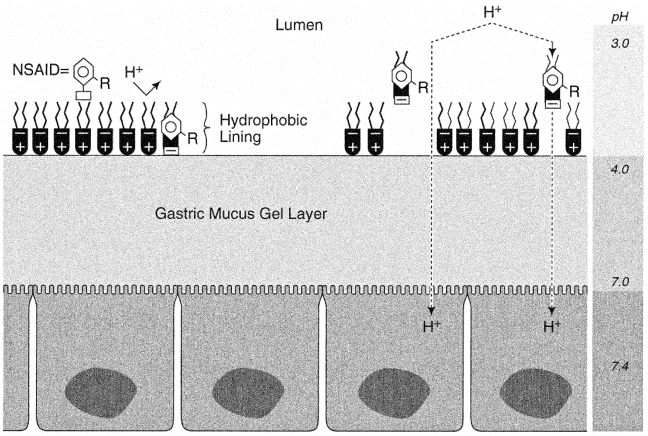

Fig. 4. Schematic model depicting the possible role of an extracellular lining of zwitterionic phospholipids in generating the hydrophobic barrier of the stomach to luminal acid, and the mechanism by which aspirin and other conventional NSAIDs may compromise that surface barrier.

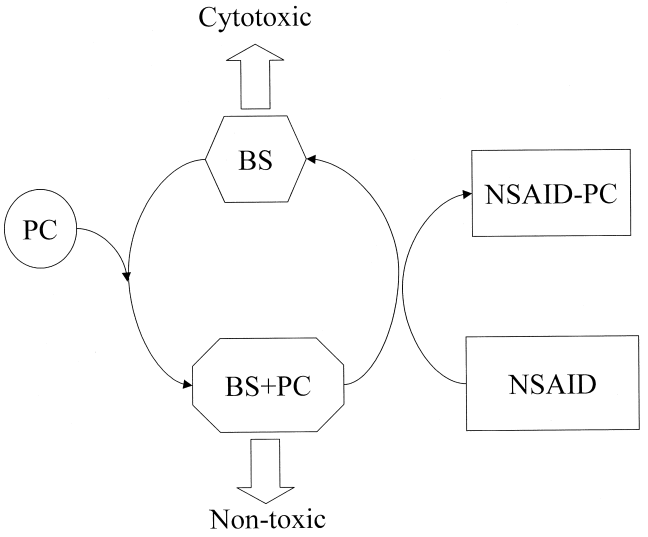

Fig. 5. Schematic model depicting potential competition between bile salts (BS) and NSAIDs for biliary PC. Increasing concentrations of NSAIDs in the bile will effectively displace PC from mixed micelles, resulting in the generation of simple bile salt micelles that are more toxic to the GI epithelium.

in rodent ulcer models [75,77]. Furthermore, similar to the case of NO-NSAIDs and unlike the COX-2 selective inhibitors, ulcer healing appeared to be accelerated significantly (compared with the effects of the unmodified NSAID) when rats with acetic acid-induced gastric ulcers were treated daily with aspirin-PC [78]. A recently published clinical trial using a randomized cross-over design has provided evidence that aspirin-PC is significantly less toxic to the human gastric mucosa than unmodified aspirin over a 4-day study period [79], and that similar to NO-NSAIDs this gastroprotective activity did not relate to an alteration in the COX-inhibitory activity of the drug.

We have also recently completed a study on rodents, which may provide an explanation as to how NSAIDs secreted into the bile possibly induce GI injury. These studies, which are described in more detail in a recently published paper [80], are based on evidence by our and other laboratories that biliary PC plays a physiologically important role in reducing the cytotoxic activity of bile salts, most likely by forming mixed micelles [81,82]. Furthermore, as schematically depicted in Fig. 5, secretion of NSAIDs into the bile abrogates this protective property, due to affinity of this family of drugs to chemically interact with PC, transforming mixed micelles back into cytotoxic bile salt micelles.

In summary, we feel that the monolithic concept that NSAIDs induce GI mucosal injury predominantly by inhibiting mucosal COX-1 activity needs to be re-examined in light of the scientific evidence. In many cases, both laboratory and clinical studies have demonstrated that the injurious action of NSAIDs and their ability to inhibit COX-1 of the gastric mucosa are not linked, leading to the possibility that these drugs may injure by a mechanism other than COX inhibition. A rather compelling case can be made that NSAIDs induce GI ulceration and bleeding primarily by topically injuring the mucosa, as they can enter the lumen via oral consumption, secretion into the bile, or both. In this commentary, we have presented a case that zwitterionic phospholipids (e.g. PC, secreted into either the mucus gel layer or bile) protect the epithelium of the GI tract from the toxic effects of noxious agents present in the lumen (HCl or bile salts). Furthermore, NSAIDs may induce GI ulceration and bleeding by associating with PC and related phospholipids, diminishing their availability to protect the GI mucosa against these luminal ulcerogens.

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