

# Phospholipid Association Reduces the Gastric Mucosal Toxicity of Aspirin in Human Subjects

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**OBJECTIVE:** In previous studies on rats, we have shown that aspirin (ASA)-induced injury to the gastric mucosa is markedly reduced or completely abolished if ASA is chemically associated with the phospholipid, phosphatidylcholine (PC). We have also shown that the protective effect of PC does not influence the ability of ASA to inhibit mucosal cyclooxygenase (COX) activity in the stomach and other tissues. We therefore sought to assess the effect of PC-associated ASA (ASA/PC) on the gastric mucosa of normal volunteers and to compare the results with the use of ASA alone.

**METHODS:** Sixteen normal healthy subjects were administered ASA or ASA/PC in a randomized, double-blind, crossover study. The subjects received ASA in a dose of 650 mg three times a day for 3 days or an equivalent dose of ASA chemically associated with PC. Endoscopy was performed at baseline and again on the morning of day 4, after the subjects had taken the final dose of the test drug. On both occasions, antral biopsy specimens were obtained for the assessment of mucosal COX activity and prostaglandin concentration.

**RESULTS:** The number (mean  $\pm$  SD) of gastric erosions seen with the ASA/PC formulation was significantly less than when ASA was used alone ( $8.7 \pm 10.7$  vs  $2.9 \pm 4.3$ ;  $p < 0.025$ ). A similar trend was seen in the duodenum but the difference was statistically not significant. The antral mucosal COX activity, as well as the level of prostaglandin 6-keto PGF<sub>1 $\alpha$</sub> , were reduced significantly (80–88%) and to a similar extent by both ASA and ASA/PC.

**CONCLUSIONS:** The present study shows that acute aspirin-induced damage to the gastric mucosa can be reduced by chemically associating ASA with PC. The mechanism of mucosal protection provided by this compound is not related to any alteration in the ability of ASA to inhibit mucosal COX activity. We believe this protection is attributable to the maintenance of the defensive hydrophobic barrier of the gastric mucosa. (Am J Gastroenterol 1999;94:1818–1822. © 1999 by Am. Coll. of Gastroenterology)

## INTRODUCTION

Aspirin (ASA) is one of the most commonly used over-the-counter drugs, with an annual consumption estimated at 40 billion tablets in 1989 (1). Aspirin use is expected to increase because of increasing public awareness of the therapeutic efficacy of ASA in preventing stroke, unstable angina, vascular thrombosis, colon cancer, and possibly Alzheimer's disease, and because of the steady increase in the average age of our society. A major problem with this trend is that aspirin and related nonsteroidal antiinflammatory drugs (NSAIDs) have the potential of damaging the gastrointestinal (GI) mucosa. NSAID use is associated with significant GI side effects, including the development of mucosal erosions and ulcers, resulting in serious and at times fatal GI bleeding. It has been estimated that NSAID users have 3–4 times greater relative risk of developing serious adverse GI events than nonusers (2). Furthermore, a retrospective study restricted to rheumatoid arthritis patients in the U.S. concluded that NSAID usage is responsible for 20,000 hospitalizations and 2600 deaths annually in this selected patient population (3). Another study focused on the long-term use of aspirin for the prevention of myocardial infarction demonstrated that a daily dose of aspirin of 1000 mg/day increased the risk for hospitalization for gastroduodenal ulceration by  $\sim$ 8-fold (4). These GI side effects cannot simply be avoided by reducing the dosage of aspirin (or other NSAIDs), as doses of aspirin as low as 40–80 mg have been reported to induce GI erosions and ulceration in susceptible individuals (5, 6).

The advent of the new selective cyclooxygenase (COX)-2 inhibitors, which appear to target the inducible COX-2 enzymes at sites of inflammation while preserving the COX-1 of the GI mucosa and their product of cytoprotective prostaglandins, may lower the incidence of GI side effects in individuals chronically taking NSAID due to inflammatory conditions (7–9). However, there remains a great need to develop a GI-safe aspirin for those taking the drug for cardiovascular risk reduction, estimated at 30–40% of total aspirin consumption (10), due to its ability to inhibit platelet COX-1 activity (11). It is also important to note that, unlike

other NSAIDs that are thought to induce GI toxicity by inhibiting the constitutive COX-1 activity of the GI mucosa, considerable evidence exists that aspirin injures the gastroduodenal mucosa, in part, by a direct topical action (12).

The gastric mucosa of a number of mammalian species, including humans, has unique hydrophobic, nonwetable surface properties that may serve as a barrier to luminal acid (13). This hydrophobic barrier property may be attributable to the ability of surface mucus cells to secrete a surfactant-like phospholipid that, in turn, is recruited to the luminal interface of the mucus gel layer (13, 14). Over the past 16 yr, our laboratory and those of others have obtained evidence that a number of damaging agents (*e.g.*, NSAIDs and *H. pylori*) rapidly attenuate the hydrophobic properties of the gastric mucosa of humans and laboratory animals, whereas this nonwetable characteristic appears to be fortified by gastroprotective agents, such as prostaglandins (13–16). Aspirin is one of the most effective agents in attenuating the hydrophobic barrier properties of the gastric mucosa, and does so in a dose- and time-dependent manner (17). Recently, we obtained evidence that aspirin and other NSAIDs may reduce surface hydrophobicity by chemically associating with and destabilizing the phospholipids that are present within and coat the mucus gel layer, with a strong preference for the zwitterionic species phosphatidylcholine (PC) (18). With this information in hand, we reasoned that this ability of aspirin and other NSAIDs to topically injure the mucosa may be prevented if the drug was chemically preassociated with synthetic or purified PC. Preclinical studies on experimental animals demonstrated a remarkable reduction in the ability of a number of NSAIDs to induce GI ulceration and bleeding when the drugs were chemically associated with PC before intragastric administration (18). We also observed that the ability of daily aspirin administration to retard the healing of experimentally induced gastric ulcers could be overcome if rats were treated with PC-associated aspirin (19). Related studies using animal models of fever and inflammation indicate that PC-associated NSAIDs had equal or enhanced therapeutic activity, compared with the use of NSAIDs alone (18, 20).

The present study was therefore carried out to determine if the damaging effect of aspirin on the gastroduodenal mucosa of human subjects could be reduced by complexing ASA with the zwitterionic phospholipid PC.

## MATERIALS AND METHODS

### *Clinical Protocol*

Normal healthy subjects of either gender who had not consumed NSAIDs during the preceding 2 wk were invited to take part in the study. Regular aspirin users and those who had consumed one or more tablets of aspirin or another NSAID during the 2-wk period before the baseline endoscopic procedure were thereby excluded from the study. After an overnight fast, a baseline endoscopy was performed and the number of mucosal erosions, if any, were noted.

Two antral biopsy specimens were obtained for analysis of mucosal COX activity and prostaglandin concentration. The subjects were then administered ASA or ASA/PC in a randomized, double-blind, crossover fashion. Each subject took 10 doses of the medication containing 650 mg of ASA per dose, predissolved in a sodium bicarbonate solution, or an equivalent dose of ASA preassociated (as outlined later) with an equimolar amount of PC (Phospholipon 90G, American Lecithin Co., Oxford) prepared daily. The subjects were instructed to take the medication three times a day (before breakfast, lunch, and dinner) for 3 days. The final dose was taken on the morning of Day 4, about 30 min before the second endoscopy. The medications were prepared fresh each day and the volunteers collected the daily supply in the morning. At endoscopy, the number and location of mucosal erosions were determined and antral biopsy specimens were obtained as before. It should be noted that the biopsy sites, which were localized in well-documented regions of the antrum (close to the pylorus) were easily identified during follow-up endoscopy and were not included when the number of erosions were counted. Similarly, the number of erosions recorded during the baseline endoscopic procedure were also excluded when the number of new erosions was calculated. After a wash-out period of 3 wk, the subjects were crossed over to the alternate medication and the protocol was repeated as described earlier. The study was approved by the Institutional Review Board of Baylor College of Medicine and an informed consent was obtained from each volunteer.

### *Chemical Association of ASA With PC*

An amount of Phospholipon 90G (containing >93% PC), equimolar to that of ASA, was dissolved in ethanol and subsequently blown-dry to a lipid film under lyophilization. At this point the ASA, which had been predissolved in 1% sodium bicarbonate, was added to the flasks containing the dried PC film, and the tubes were vortexed for 10 min, resulting in the final ASA/PC suspension (pH, 5.4) that was administered to the subjects.

### *Prostaglandin Analysis and Assessment of Mucosal COX Activity*

Antral mucosal biopsy tissue was weighed, minced, and either incubated in 50 mmol/L Tris buffer (pH, 7.4) containing arachidonic acid (20  $\mu$ mol/L) for 10 min at 37°C in a shaking water bath, followed by microfuge centrifugation (10,000 *g* for 3 min) and collection of the supernatant, to assess COX activity; or immediately extracted in methanol, dried using a Speed Vac (RVT-100) concentrator system (Farmingdale, NY) and then redissolved in buffer, to assess mucosal prostaglandin concentration. Both samples were analyzed for 6-keto PGF<sub>1 $\alpha$</sub>  (a stable metabolite of PGI<sub>2</sub>) by a highly sensitive radioimmunoassay that has been described previously (21, 22).

### Statistical Analysis

A Wilcoxon Signed Rank test was employed to determine the presence of statistically significant differences in gastroduodenal erosions between groups, with a  $p < 0.05$  being considered significant. A two-way Analysis of Variance (ANOVA) was also employed to determine the presence of statistically significant differences in mucosal COX activity and prostaglandin concentration between groups, with  $p < 0.05$  being considered significant.

## RESULTS

### Gastroduodenal Erosions

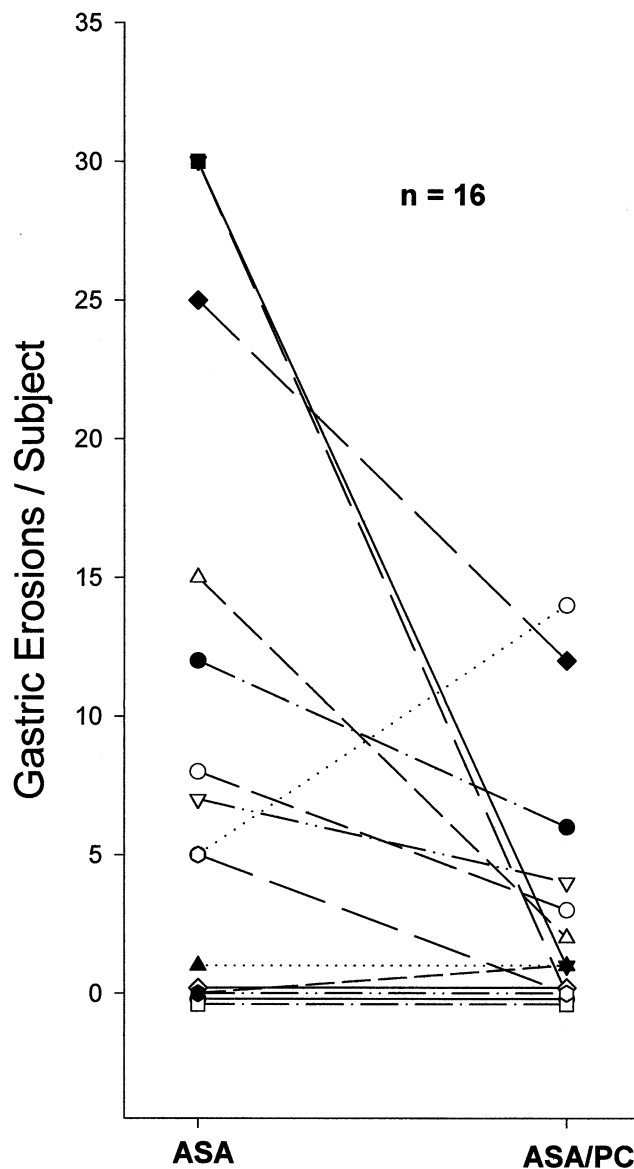
Sixteen healthy volunteers, six men and 10 women, took part in the study. The mean ( $\pm$ SD) age of the subjects was  $41.1 \pm 7.3$  yr. The results of endoscopic findings are depicted in Figure 1. It can be seen that nine of the 16 subjects (56%) had at least five gastric erosions each after a 4-day course of aspirin therapy, with a range of five to 30 gastric lesions/subject. Erosions were almost always seen in the antrum, whereas erosions in the gastric body were generally observed only in the more aspirin-intolerant subjects (those with  $\geq 25$  erosions/subject). Furthermore, eight of the nine (89%) of these aspirin-intolerant individuals experienced fewer gastric lesions after completing the ASA/PC arm of the study. The mean number of gastric erosions per subject ( $\pm$ SD) seen after administration of aspirin was significantly greater than the number of gastric erosions observed in these same subjects treated with the ASA/PC formulation ( $8.75 \pm 10.76$  vs  $2.81 \pm 4.34$ ;  $p = 0.025$ ). Similarly, the mean number of duodenal lesions per subject was greater after ASA use ( $1.13 \pm 2.16$ ), compared with ASA/PC ( $0.19 \pm 0.75$ ); however, this difference in duodenal erosions between groups failed to reach statistical significance.

### Mucosal Prostaglandin Concentration and COX Activity

Analysis of antral mucosal 6-keto  $\text{PGF}_{1\alpha}$  levels, incubated with exogenous  $20 \mu\text{mol/L}$  arachidonic acid, revealed that a 4-day course of ASA inhibited the COX activity by 83–88% in comparison to baseline values, regardless if the drug was chemically associated with PC or not (Fig. 2). Similarly, the endogenous concentration of 6-keto  $\text{PGF}_{1\alpha}$  in the antral mucosa was inhibited by 80–82% in response to treatment with either ASA or ASA/PC (Table 1).

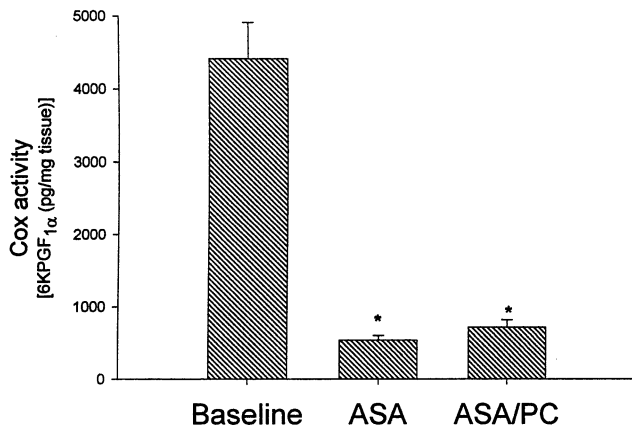
## DISCUSSION

The present clinical study confirms our findings in rats (18) that chemical association of aspirin with PC, a zwitterionic phospholipid, significantly reduces the gastric mucosal toxicity of aspirin when used over a 4-day study period. Similar to reports in the literature that indicate that 30–60% of individuals taking aspirin and other NSAIDs develop gastroduodenal erosions (23, 24), 56% of our healthy volunteers demonstrated an intolerance to aspirin, having five or more gastric erosions at the end of the 4-day study period.



**Figure 1.** The number of gastric erosions induced in 16 healthy subjects by a 4-day course of aspirin (ASA, 650 mg  $3 \times$  day) is significantly reduced if the subjects take an equivalent dose of ASA, chemically associated with an equimolar amount of phosphatidylcholine (PC).

This accounts for the large variability in the data on gastric injury (as the number of gastric erosions/subject in the subject population ranged from 0 to  $>30$ ). Furthermore, the protective effect of the phospholipid occurred in eight of the nine aspirin-intolerant subjects, and was most apparent in those who were most sensitive to the injurious actions of the NSAID. A similar trend was observed with respect to protection of the duodenal mucosa, although the difference failed to reach statistical significance. We believe that the protective effects of our test formulation relate to the observation that preassociation of PC with the NSAID reduces aspirin's ability to interact with and destabilize the intrinsic phospholipids of the mucus gel layer. Our laboratory has



**Figure 2.** The COX activity of antral mucosal biopsy tissue, as determined by measuring the generation of 6-keto  $\text{PGF}_{1\alpha}$  upon incubating the tissue in  $20 \mu\text{mol/L}$  arachidonic acid, was comparably inhibited by 83–88% after the subjects were treated with either ASA or the ASA/PC formulation.

previously reported evidence that these extracellular phospholipids contribute to the surface hydrophobicity of the gastroduodenal mucosa, which appears to provide a barrier to the back-diffusion of luminal acid into the tissue (13, 14).

Our study also indicates that this protective action of the phospholipid is not due to a decrease in the ability of the NSAID to inhibit gastric mucosal COX activity, as the generation of 6-keto  $\text{PGF}_{1\alpha}$  in the gastric mucosa was reduced by a comparable amount with both ASA and ASA/PC. These findings are in support of our preclinical data (18, 20), and indicate that the low GI toxicity of the ASA/PC formulation is not attributable to a lower bioavailability of the NSAID or an attenuation in its ability to inhibit the COX-1 activity of the gastroduodenal mucosa. These clinical findings, together with our laboratory studies, are thereby consistent with the concept that, unlike other NSAIDs, aspirin primarily induces gastroduodenal erosions in both humans and laboratory animals by topically injuring the mucosa through a mechanism independent of its ability to inhibit COX activity (12). Interestingly, it has recently been reported that covalent bonding of a nitric oxide group to a number of NSAIDs also has the capacity to markedly reduce the drugs' GI toxicity, although their ability to inhibit mucosal COX activity is unimpaired (12, 25).

In conclusion, the present study suggests that the ASA/PC complex may be an effective and safe means of decreasing or eliminating the topical injurious action of aspirin on the

**Table 1.** Effect of a 4-Day Course of ASA or ASA/PC on the Endogenous Antral Mucosal Concentration of 6-keto  $\text{PGF}_{1\alpha}$

Group	Antral Mucosal 6-keto $\text{PGF}_{1\alpha}$ Concentration (pg/mg Wet Weight)
Baseline	$1338 \pm 348$
ASA	$268 \pm 79^*$
ASA/PC	$256 \pm 29^*$

\*  $p < 0.05$  vs baseline value. There was no significant difference between ASA vs ASA/PC.

gastroduodenal mucosa. It is our contention that by chemically associating aspirin with PC, the topical irritant actions of the NSAID are abolished or reduced by maintaining the protective surface hydrophobic properties of the gastroduodenal mucosa. We emphasize that the present results relate specifically to a therapeutic approach to reduce the topical gastric toxicity of aspirin where the drug is administered for a relatively short period of time. Future more chronic studies are planned to evaluate the clinical relevance of phospholipid association in preventing gastroduodenal ulceration caused by more extensive exposure to aspirin and other NSAIDs.

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