

# Nonsteroidal Anti-inflammatory Drug and Phospholipid Prodrugs: Combination Therapy With Antisecretory Agents in Rats

LENARD M. LICHTENBERGER, CARLOS ULLOA, JIMMY J. ROMERO, AMY L. VANOUS, PAUL A. ILLICH, and ELIZABETH J. DIAL

Department of Integrative Biology, University of Texas-Houston Medical School, Houston, Texas

See editorial on page 1145.

**Background & Aims:** The gastrointestinal side effects of nonsteroidal anti-inflammatory drugs (NSAIDs) are reduced by antisecretory agents. The effects of combination therapy on the gastrointestinal toxicity and therapeutic activity of free and phospholipid-associated NSAIDs were investigated in rats. **Methods:** Fasted rats, pretreated with either saline or an antisecretory dose of omeprazole, ranitidine, or cimetidine, were intragastrically administered saline, aspirin, or indomethacin. In ulcer models, gastric lesions in aspirin-treated rats and intestinal bleeding in indomethacin-treated rats were measured. For antipyretic and analgesic activity, rectal body temperature in febrile rats and the rats' pain sensitivity to pressure applied to an inflamed limb were measured, respectively. **Results:** NSAID-induced gastrointestinal ulceration and bleeding were reduced in rats pretreated with antisecretory agents and abolished in rats administered phospholipid-associated NSAIDs in combination with inhibitors of acid secretion. The antipyretic and analgesic activity of both NSAIDs was attenuated in rats pretreated with an antisecretory agent. This pH-dependent block in therapeutic activity was overcome if the NSAID was preassociated with a phospholipid to enhance the drug's lipophilic characteristics. **Conclusions:** Combination therapy of antisecretory agents and NSAIDs, chemically associated with phospholipids, has distinct advantages with regard to both low gastrointestinal toxicity and restored therapeutic activity.

The consumption of nonsteroidal anti-inflammatory drugs (NSAIDs) is considered a risk factor in the development of peptic ulcer disease because studies indicate that the incidence of developing gastroduodenal ulcers and/or gastrointestinal (GI) bleeding is significantly increased in subjects taking NSAIDs.<sup>1,2</sup> We have recently reported that the ability of NSAIDs to induce GI bleeding and ulceration in rats can be reduced if the drugs are chemically associated with zwitterionic phospholipids.<sup>3</sup> This form of synthetic bonding presumably acts by lim-

iting the availability of NSAIDs to interact with and neutralize the surface activity of intrinsic phospholipids that line the mucus gel layer of the upper GI tract.

Another approach advocated to reduce the GI toxic effects of these drugs is to concomitantly administer an NSAID and one of several potent antisecretory agents to neutralize gastric acidity.<sup>4-8</sup> Although this seems to be an effective and promising strategy to reduce some of the GI side effects of NSAIDs, a question that needs to be addressed is whether inhibition of gastric acidity affects the bioavailability and therapeutic activity of this powerful family of drugs. This possibility is worth considering because aspirin and many other NSAIDs are weak acids and are thought to primarily enter circulation by absorption across the gastroduodenal epithelium in their nondissociated, lipidic state.<sup>9</sup> Thus, neutralizing gastric juice pH would markedly reduce the lipophilic nature of these molecules and alter their site of absorption to more distal regions of the GI tract that contain a different complement of digestive enzymes.

In the present study, rat model systems were used to investigate the effects of both proton pump inhibitors and H<sub>2</sub>-receptor antagonists on the short-term GI toxicity and antipyretic and analgesic activity of aspirin and indomethacin. Parallel experiments were performed with these NSAIDs coupled to phospholipids to determine if this may be an effective strategy to increase the bioavailability of the NSAID in the presence of an antisecretory agent while providing enhanced protection against the drugs' injurious actions.

## Materials and Methods

### Animals

Male Sprague-Dawley rats weighing 150-175 g were purchased from Harlan Sprague-Dawley Inc. (Indianapolis,

*Abbreviations used in this paper:* DPPC, dipalmitoylphosphatidylcholine; ED<sub>50-80</sub>, 50%-80% effective dose; GI, gastrointestinal; L-NAME, N<sup>ε</sup>-nitro-L-arginine methyl ester.

© 1996 by the American Gastroenterological Association  
0016-5085/96/\$3.00

IN) and housed in our institution's Animal Care Center 5–10 days before study, during which time they had ad libitum access to water and Harlan Teklab F-6 Rodent Diet (Madison, WI). All animal protocols had been scrutinized and approved by our institution's Animal Welfare Committee, and they met or exceeded National Institutes of Health guidelines for treatment and welfare of laboratory animals.

### Gastric pH

Fasted rats were injected intraperitoneally with saline (control) or various doses of omeprazole (Astra Hässle AB, Mölndal, Sweden), cimetidine, or ranitidine (Sigma Chemical Co., St. Louis, MO). One hour later, the rats were anesthetized with ether; after a midline incision, the stomachs were exteriorized, and 2 mL of H<sub>2</sub>O was injected into the gastric lumen and collected for pH analysis.

### Ulcer Models and Chemical Association of NSAIDs With Dipalmitoylphosphatidylcholine

Rats were fasted overnight and injected intraperitoneally with an acid-inhibitory dose of omeprazole (400  $\mu$ mol/kg or 140 mg/kg), ranitidine (5 mg/kg), or cimetidine (50 mg/kg). One hour later, the rats were intragastrically administered a 50%–80% effective dose (ED<sub>50–80</sub>) of aspirin (20 mg/kg) and challenged 10 minutes later with an intragastric dose of 0.6N HCl as outlined previously.<sup>3</sup> In this and all subsequent experiments, the NSAIDs were administered either alone or chemically associated with an equimolar concentration of dipalmitoylphosphatidylcholine (DPPC). The NSAID-DPPC complex was prepared by initially dissolving the required amount of DPPC in chloroform, followed by exhaustive overnight evaporation of the organic solvent under vacuum as described previously.<sup>3</sup> The NSAID salt, which was dissolved in water and subsequently titrated to the desired pH, was added to the tube containing the lipid film and sonicated for 15 minutes in a bath-type sonicator (Laboratory Supplies Co. Inc., Hicksville, NY) as outlined previously.<sup>3</sup> Sixty minutes after administration of aspirin with or without DPPC, the rats were killed by CO<sub>2</sub> asphyxiation, their stomachs were removed, and gastric lesions were scored in accordance to a previously described method by an individual unaware of the treatment conditions.<sup>3</sup>

The second animal model was based on a recently described method<sup>3</sup> in which fasted rats were administered N<sup>G</sup>-nitro-L-arginine methyl ester (L-NAME, 20 mg/kg intraperitoneally) 1 hour before and 3 and 6 hours after the animals received indomethacin intragastrically with or without DPPC at a dose of 10 mg/kg or saline (control). In this experiment, the antisecretory agents were administered intraperitoneally 90 minutes before indomethacin (or saline) treatment. Eighteen hours after administration of the NSAID (or saline), the rats were killed by CO<sub>2</sub> asphyxiation. The distal half of the intestine was flushed with 10 mL of saline, which in turn was collected for hemoglobin analysis to provide an estimate of GI bleeding as outlined previously.<sup>3</sup>

### Antipyretic Activity

The antipyretic activity of NSAIDs was evaluated and compared in conscious rats who were made febrile by a subcutaneous injection of 2 g/kg brewers' yeast and fasted thereafter according to a method described by Adams et al.<sup>3,10</sup> Eighteen hours later, the fasted febrile rats (body temperature increase, 0.5–1.0°C) were injected with an antisecretory agent or an equivalent volume of vehicle was injected by the same route. One hour later, the rats were treated intragastrically with 1 mL of an approximate ED<sub>50–80</sub> of either aspirin (18 mg/kg) or indomethacin (4 mg/kg) alone or the drugs preassociated with DPPC (adjusted to a pH of 7.0). Rectal body temperature was monitored over the subsequent 4-hour period.

### Analgesic Activity

We assessed the ability of NSAIDs to reduce the hyperalgesia (i.e., increased sensitivity to pain) associated with an inflamed hind paw (induced 4 days earlier by the injection of 0.05 mL complete Freund's adjuvant). A modified version of the Randall–Selitto test was used to assess pain sensitivity of each hind paw.<sup>3,11,12</sup> Rats were restrained in Plexiglas tubes, and external pressure was applied sequentially to the uninflamed and inflamed hind paws (0–250 g at a rate of 16 g/s with an Analgesymeter (Life Sciences Institute, Woodland Hills, CA) under the control of an observer unaware of the treatment groups. Pain pressure threshold was defined as the pressure at which an animal exhibits paw withdrawal. In these studies, rats were fasted overnight and pretreated with an antisecretory agent or saline 1 hour before receiving an ED<sub>50–80</sub> of the NSAID alone (10 mg/kg aspirin, 4 mg/kg indomethacin) or the NSAID preassociated with DPPC as described above. Two hours later, pain pressure thresholds were assessed on both hind paws. The dosing regimen described above was repeated at 6 and 24 hours, and the sensitivity to externally applied pressure was measured 2 hours after the animals received the last NSAID dosage.

The results were analyzed using the ANOVA procedure. If the ANOVA procedure yielded a significant interaction between variables, post ad hoc comparisons were made with Fisher's least-squares difference test ( $P < 0.05$ ). Data are expressed as mean  $\pm$  SEM, with  $P$  values of  $\leq 0.05$  considered statistically significant.

### Results

Table 1 shows that, in conscious rats, gastric acid secretion was maximally inhibited by the injection of omeprazole at a dose of 400  $\mu$ mol/kg, with gastric pH not being significant from neutrality. The H<sub>2</sub>-receptor blockers, ranitidine and cimetidine, at doses of 5 and 50 mg/kg, respectively, were somewhat less efficacious in inhibiting gastric acid secretion in the rats, increasing the gastric pH to values of  $>6.0$  in 1–2 hours.

Figure 1 shows that aspirin's ability to induce acute gastric lesions was reduced to a variable degree in rats pretreated with an antisecretory agent (with omeprazole

**Table 1.** Ability of Antisecretory Agents to Neutralize Gastric Juice pH in Rats

Groups	Dose	Gastric juice pH
Saline	0	3.45 ± 0.05
Omeprazole <sup>a</sup>	40 µmol/kg	4.15 ± 0.60
	400 µmol/kg	7.21 ± 0.15 <sup>c</sup>
Ranitidine <sup>b</sup>	5 mg/kg	6.03 ± 0.27
	20 mg/kg	6.30 ± 0.24 <sup>c</sup>
Cimetidine <sup>b</sup>	20 mg/kg	3.58 ± 0.46
	50 mg/kg	6.13 ± 0.32 <sup>c</sup>

NOTE. Fasted rats were injected intraperitoneally with omeprazole, ranitidine, cimetidine, or saline. Two hours later, a laparotomy was performed with the rats under ether anesthesia. Clamps were then placed at the esophageal and duodenal ends, and 2 mL of water was injected through the gastric wall into the lumen. After mixing, the gastric fluid was collected for pH analysis.

<sup>a</sup>Omeprazole was suspended in 1 part 10 mg/dL polyethylene glycol in 5 parts 18 mmol/L NaHCO<sub>3</sub>.

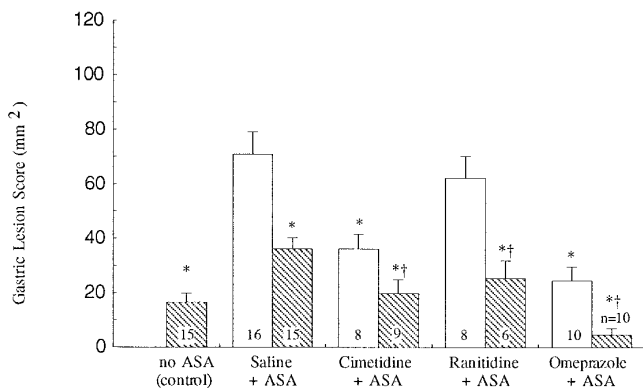
<sup>b</sup>Ranitidine and cimetidine were solubilized in distilled H<sub>2</sub>O.

<sup>c</sup>P < 0.05 vs. gastric juice pH of control rats injected with saline.

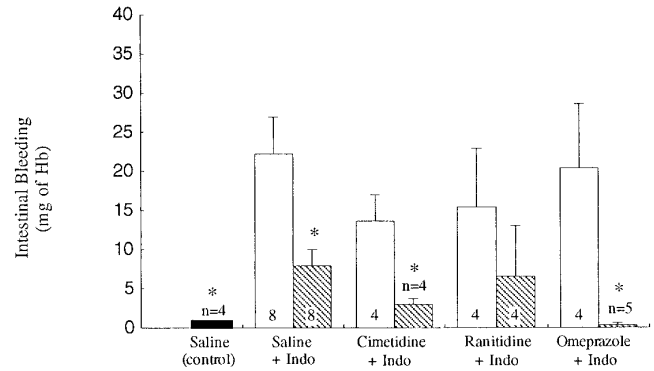
and cimetidine being most protective) or if the NSAID was chemically associated with DPPC, confirming results of previous studies. Interestingly, pretreatment of rats with any one of the antisecretory agents before intragastric administration of phospholipid-complexed aspirin afforded the animals full protection against NSAID-induced gastric lesion in this ulcer model.

Figure 2 shows that the combination therapy of antisecretory agents with indomethacin-DPPC proved superior to either the acid inhibitor or the phospholipid alone in the prevention of acute NSAID-induced GI bleeding during an 18-hour period.

Figure 3 shows that the antipyretic activity of aspirin,



**Figure 1.** Ability of omeprazole and the H<sub>2</sub>-receptor antagonists cimetidine and ranitidine to protect against short-term aspirin-induced gastric ulcer formation. Note that NSAID-induced lesion formation was prevented if combination therapy was performed with the aspirin-DPPC complex (▨) and one of the antisecretory agents. □, Aspirin (ASA). \*P < 0.05 vs. saline plus aspirin; †P < 0.05 comparing aspirin vs. aspirin-DPPC for each antisecretory agent. Numbers inside bars indicate number of animals per group.

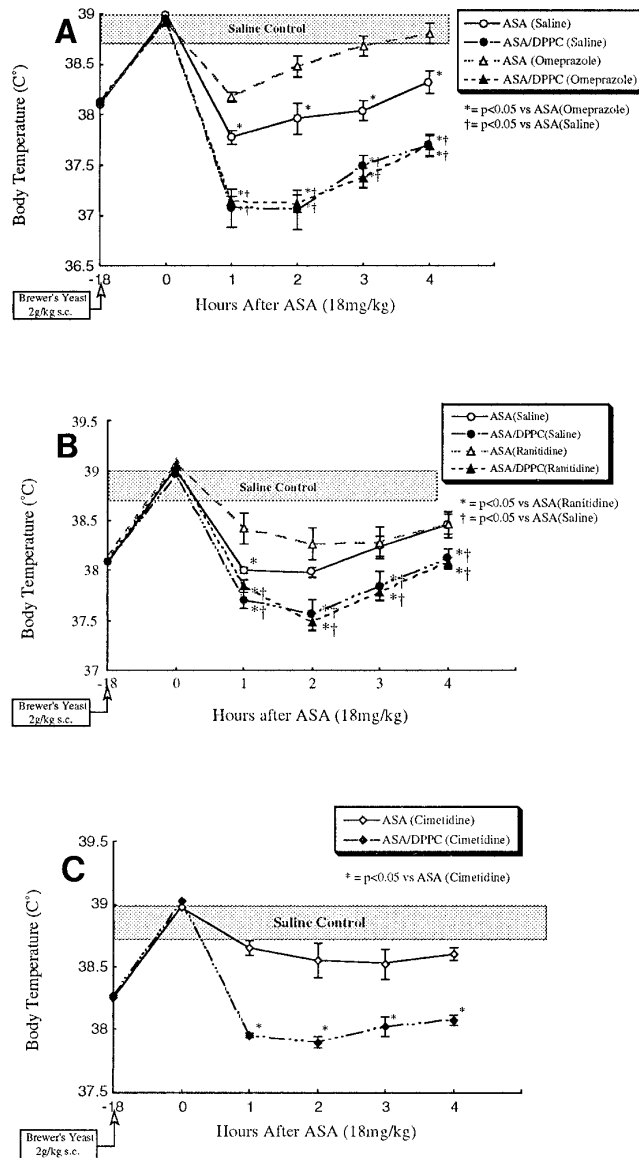


**Figure 2.** Ability of omeprazole and the H<sub>2</sub>-receptor antagonists to protect against indomethacin-induced GI bleeding in rats pretreated and posttreated with L-NAME. As in the case of aspirin, superior protection against NSAID-induced GI injury was obtained in rats pretreated with antisecretory agents before challenge with the indomethacin-DPPC complex (▨). □, Indomethacin (Indo); \*P < 0.05 vs. saline plus indomethacin; †P < 0.05 comparing aspirin vs. aspirin-DPPC for each antisecretory agent. Numbers inside bars indicate number of animals per group.

administered at a dose of 18 mg/kg, was clearly attenuated if the rats were pretreated with antisecretory doses of omeprazole, ranitidine, or cimetidine. However, the antisecretory agents' ability to attenuate aspirin's antipyretic activity was completely reversed if the NSAID was preassociated with DPPC to increase the drug's lipophilic characteristics. Figure 4 shows that the proton pump inhibitor omeprazole had a similar ability to reduce the antipyretic activity of indomethacin, administered at a dose of 4 mg/kg, which once again could be overcome if the NSAID was administered as a complex with DPPC.

Figure 5A and B shows that the analgesic activity of both aspirin and indomethacin was similarly attenuated if rats were pretreated with an antisecretory agent. Under control conditions, the pain pressure threshold of the inflamed paw increased approximately twofold from 40–60 to 90–100 mm Hg 2 hours after rats were administered the ED<sub>50–80</sub> of either NSAID alone. This analgesic action of both aspirin and indomethacin was clearly reduced or eliminated if the rats were pretreated with one of the potent antisecretory agents (Figure 5, compare open bars). Furthermore, the analgesic activity of both aspirin and indomethacin could be restored in rats pretreated with an inhibitor of gastric acid secretion if the NSAIDs were administered in the lipid-associated state (Figure 5, compare shaded bars with open bars for each antisecretory agent). An analgesic activity pattern very similar to that described above was observed 26 hours after rats received three consecutive treatments of aspirin or indomethacin with and without DPPC (at 0, 6, and 24 hours) preceded 1 hour earlier with an injection of

an antisecretory agent. Once again, the NSAID-induced analgesia was significantly reduced by pretreatment with one of the three antisecretory agents, which could be overcome if the NSAIDs were chemically associated with DPPC. Importantly, at both time points in the absence of one of the above antisecretory agents, both the antipyretic and analgesic activities of aspirin and indomethacin were at least as good and in most cases enhanced when the drug was complexed to DPPC compared with activity observed with the NSAID alone (Figure 5, compare shaded bars with open bars for rats treated with saline).

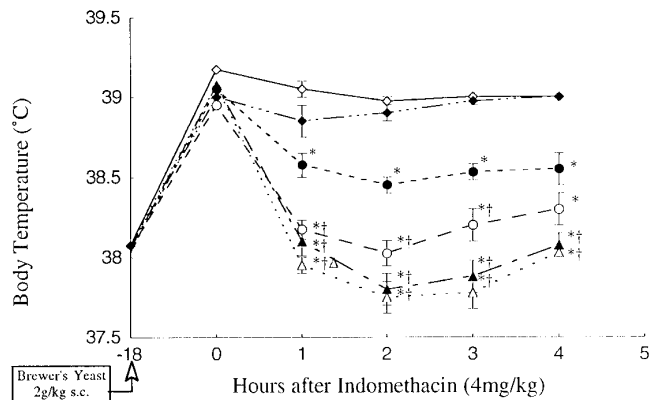


**Figure 3.** Antipyretic activity of aspirin, intragastrically administered at a dose of 18 mg/kg, is attenuated if febrile rats are treated with (A) omeprazole (400  $\mu$ mol/kg), (B) ranitidine (5 mg/kg), or (C) cimetidine (50 mg/kg). This block in antipyretic activity, induced by pretreating the rats with antisecretory agents, could be overcome if aspirin was administered as an equimolar complex with DPPC.

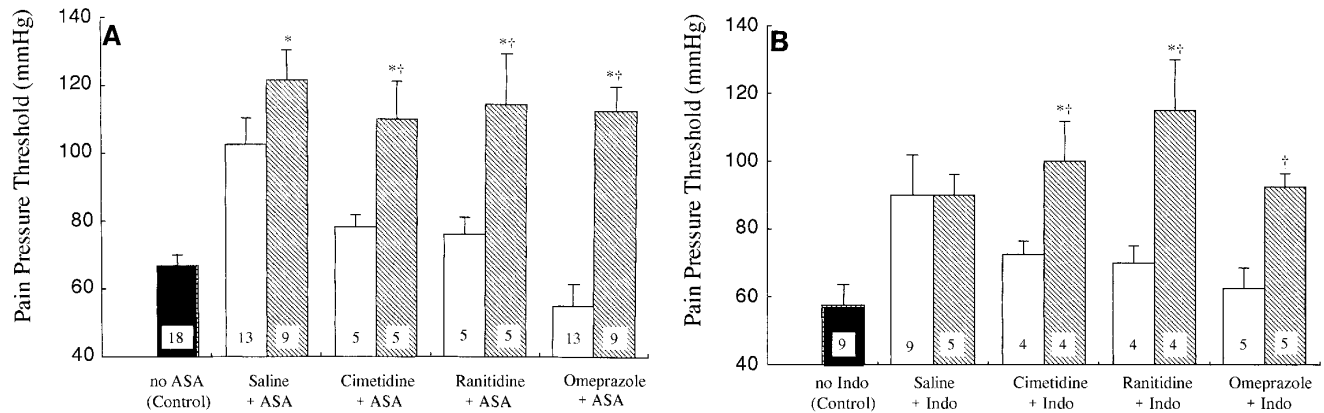
### Discussion

Proton pump inhibitors and H<sub>2</sub>-receptor antagonists are presently recommended for both the prevention and treatment of gastroduodenal ulcers associated with NSAID use on the basis of encouraging clinical findings.<sup>4-8</sup> Using rodent model systems, we obtained confirmatory evidence that pretreatment of rats with antisecretory doses of omeprazole, ranitidine, and cimetidine was moderately effective in reducing GI injury and bleeding induced by short-term exposure to aspirin or indomethacin. Building on recent reports from our laboratory that the GI injurious actions of NSAIDs can be reduced if the drugs are chemically associated with the zwitterionic phospholipid DPPC,<sup>3</sup> we also tested the GI toxicity of the above potent antisecretory agents in combination with phospholipid-associated NSAIDs. The results clearly indicate that this strategy proved the most effective in eliminating the short-term GI side effects of both aspirin and indomethacin.

The results of the present study based on rodent model systems indicate that the powerful antisecretory agents omeprazole, ranitidine, and cimetidine seem to attenuate the therapeutic activity of anionic NSAIDs to reduce fever, pain, and inflammation. This apparent reduction in bioavailability was best observed when the antisecretory agents were administered at a dose to increase gastric juice pH to >6.0 and when the NSAIDs were administered at an ED<sub>50-80</sub> to induce antipyretic and anti-inflammatory or analgesic activity. These laboratory findings thereby suggest that although combination therapy of antisecretory agents with NSAIDs seems to effectively



**Figure 4.** Antipyretic activity of indomethacin, intragastrically administered at a dose of 4 mg/kg, was also attenuated if febrile rats were pretreated with omeprazole (400  $\mu$ mol/kg) 1 hour before the NSAID. This block could similarly be overcome if indomethacin was administered as an equimolar complex with DPPC.  $\diamond$ , Saline (vehicle);  $\blacklozenge$ , saline (omeprazole);  $\circ$ , indomethacin (vehicle);  $\bullet$ , indomethacin (omeprazole);  $\triangle$ , indomethacin-DPPC (vehicle);  $\blacktriangle$ , indomethacin-DPPC (omeprazole). n = 4/group. \*P < 0.05 vs. saline;  $\dagger$ P < 0.05 vs. indomethacin (omeprazole);  $\Delta$ P < 0.05 vs. indomethacin (vehicle).



**Figure 5.** The ability of (A) aspirin ( $\square$ ; 10 mg/kg) and (B) indomethacin ( $\square$ ; 4 mg/kg) to increase the pain pressure threshold of an inflamed paw over saline-treated control values at 2 hours was eliminated if rats were pretreated with an antisecretory dose of cimetidine, ranitidine, or omeprazole. This block in NSAID-induced analgesia could be overcome if the NSAID was preassociated with an equimolar concentration of DPPC ( $\text{▨}$ ). The pain pressure threshold of the uninflamed paw of saline-treated control rats was  $11.2 \pm 5$  mm Hg. (A) ASA, aspirin. \* $P < 0.05$  vs. saline plus aspirin; † $P < 0.05$  comparing aspirin vs. aspirin-DPPC for each antisecretory agent. (B) Indo, indomethacin. \* $P < 0.05$  vs. saline plus indomethacin; † $P < 0.05$  comparing indomethacin vs. indomethacin-DPPC for each antisecretory agent. Numbers inside bars indicate number of animals per group.

reduce the GI toxicity of NSAIDs as a result of neutralization of gastric acidity, it may be contraindicated in clinical situations in which relief from pain, inflammation, and fever is needed. Accordingly, we believe that clinical studies are now warranted to determine whether similar drug interactions occur in humans.

The mechanism by which omeprazole, ranitidine, and cimetidine blocks the therapeutic activity of both aspirin and indomethacin is presently uncertain but most likely relates to their common ability to neutralize gastric acidity and block the acid-dependent conversion of the NSAIDs into their membrane-permeable lipidic state. This explanation is based on evidence indicating that orally administered anionic NSAIDs are absorbed across the upper GI tract primarily in their undissociated lipidic state.<sup>9</sup> Thereby, pharmacological neutralization of the gastric juice to  $\text{pH} > \text{pK}_a$  of the NSAID would limit the absorption of the drugs across the gastroduodenal mucosa. This block in the gastric absorption of the drugs would result in the movement of the NSAIDs into, and possibly their absorption from, lower regions of the small intestine where they could be exposed to metabolism by both pancreatic and/or brush border enzymes.

We have previously reported that the short-term GI toxicity of NSAIDs is reduced if the drugs are administered as a preformed complex with DPPC; we also obtained preliminary evidence that the antipyretic and anti-inflammatory activity of the complex was superior to that of the NSAID alone.<sup>3</sup> We speculated that this apparent enhancement in therapeutic activity may be attributable to an increase in the lipid permeability and solubility of the phospholipid-associated NSAID and provided some

in vitro evidence to support this possibility. It was predicted that ionic binding between the DPPC and the NSAID shields the NSAID from undergoing pH-dependent changes in charge and that, unlike the free NSAID, the complex would remain lipophilic even as the intragastric pH approached neutrality. The experimental evidence of the present study seems to support this possibility because the block in the NSAIDs' antipyretic and anti-inflammatory/analgesic activities observed with the three potent antisecretory test agents could be overcome if the NSAIDs were administered to rats as a preformed complex with DPPC. It was also interesting that even in the absence of the antisecretory agents, both NSAIDs, when administered as a lipidic complex, seemed to have equivalent or greater therapeutic efficacy to that of the NSAID alone in reducing fever and pain; these findings confirm both our initial results and those of a recent extensive study on the ability of DPPC to enhance the therapeutic activity of NSAIDs.<sup>3,12</sup>

In summary, we have shown in rodent model systems that the therapeutic activity of NSAIDs may be compromised if administered together with potent inhibitors of gastric acid secretion. Another approach is to administer the NSAID as a preformed complex with a zwitterionic phospholipid, such as DPPC, either alone or together with an inhibitor of gastric acid secretion. These experimental ulcer studies indicate that this form of combination therapy would be effective in eliminating the GI side effects of the drug while maintaining or enhancing the drugs' therapeutic activity. Studies are presently under way to determine if chemical association of NSAIDs with DPPC may affect the drugs' GI toxicity and thera-

peutic activity by altering the NSAIDs' pharmacokinetics and their ability to inhibit selective isoforms of cyclooxygenase.

## References

1. Allison MC, Howatson AG, Torrance CJ, Lee FD, Russell RI. Gastrointestinal damage associated with the use of non-steroidal anti-inflammatory drugs. *N Engl J Med* 1992;327:749-754.
2. Kurata JH, Abbey DE. The effect of chronic aspirin use on duodenal and gastric ulcer hospitalizations. *J Clin Gastroenterol* 1990;12:260-266.
3. Lichtenberger LM, Wang Z-M, Romero JJ, Ulloa C, Perez JC, Giraud M-N, Barreto JC. Non-steroidal anti-inflammatory drugs (NSAIDs) associate with zwitterionic phospholipids: insight into the mechanism and reversal of NSAID-induced gastrointestinal injury. *Nature Med* 1995;1:154-158.
4. Scheiman JM, Behler EM, Loeffler KM, Elta GH. Omeprazole ameliorates aspirin-induced gastroduodenal injury. *Dig Dis Sci* 1994;39:97-103.
5. Daneshmend TK, Stein AG, Bhaskar NK, Hawkey CJ. Abolition by omeprazole of aspirin-induced gastric mucosal injury in man. *Gut* 1990;31:514-519.
6. Ekström P, Carling L, Wetterhus S, Wingren PE. Omeprazole reduces the frequency of gastroduodenal lesion and dyspeptic symptoms during NSAID therapy (abstr). *Gastroenterology* 1995;108:A87.
7. Agrawal NM. Epidemiology and prevention of non-steroidal anti-inflammatory drug effects in the gastrointestinal tract. *Br J Rheumatol* 1995;34 (Suppl 1):5-10.
8. Hudson N, Taha AS, Russell RI, Sturrock RG, Tyre P, Cottrell J, Mann JG, Swannell A, Hawkey CJ. High dose famotidine as healing and maintenance treatment for NSAID-associated gastroduodenal ulceration (abstr). *Gastroenterology* 1995;108:A117.
9. McCormack K, Bryne K. Classical absorption theory and the development of gastric mucosal damage associated with non-steroidal anti-inflammatory drugs. *Arch Toxicol* 1987;60:261-269.
10. Adams SS, Hebborn P, Nicholson JS. Some aspects of the pharmacology of ibufenac, a non-steroidal anti-inflammatory agent. *J Pharm Pharmacol* 1968;20:305-312.
11. Randall LO, Selitto JJ. A method for measurement of analgesic activity of inflamed tissue. *Arch Int Pharmacodyn* 1957;111:409-419.
12. Lichtenberger LM, Ulloa C, Vanous AL, Romero JJ, Dial EJ, Ilich PA, Walters ET. Zwitterionic phospholipids enhance aspirin's therapeutic activity as demonstrated in rodent model systems. *J Pharmacol Exp Ther* 1996;277:1221-1227.

---

Received March 28, 1996. Accepted May 24, 1996.

Address requests for reprints to: Lenard M. Lichtenberger, Ph.D., Department of Integrative Biology, University of Texas-Houston Medical School, P.O. Box 20708, Houston, Texas 77225. Fax: (713) 794-1349.

Supported in part by National Institutes of Health grant DK 33239. The authors thank Drs. Björn Wallmark and Pia Lorentzon from Astra Hässle AB (Möndal, Sweden) for providing us with omeprazole.