

# ASSOCIATION OF PHOSPHATIDYLCHOLINE AND NSAIDS AS A NOVEL STRATEGY TO REDUCE GASTROINTESTINAL TOXICITY

Lenard M. Lichtenberger<sup>1</sup>, Melisa Barron<sup>2</sup> and Upendra Marathi<sup>2</sup>

<sup>1</sup>Department of Integrative Biology & Pharmacology, The University of Texas Health Science Center, Houston, Texas;  
<sup>2</sup>PLx Pharma Inc., Houston, Texas, USA

## CONTENTS

Summary .....	877
Introduction .....	878
Rationale for the development of PC-NSAIDs .....	878
Gastric surface hydrophobicity: its measurement and perturbation by NSAIDs .....	879
Current approaches and alternatives to mitigate NSAID gastric toxicity .....	880
Phosphatidylcholine-associated NSAIDs under development .....	881
Preclinical evaluation .....	881
Clinical evaluation .....	886
Conclusions .....	887
References .....	888

## SUMMARY

*Nonsteroidal anti-inflammatory drugs (NSAIDs) are highly effective drugs that inhibit pain and inflammation, and perhaps due to the role of inflammation in the underlying etiology, NSAIDs have also demonstrated efficacy in*

*reducing a patient's risk of developing a number of cancers and neurological diseases (e.g. Alzheimer's disease). The utility of these powerful drugs is limited due to their gastrointestinal (GI) side-effects, notably peptic ulceration and GI bleeding which is briefly reviewed here. We also describe the barrier property of the GI mucosa and how it is affected by NSAIDs, as it is our position that disruption of the surface barrier is an important component in the drugs' pathogenesis, in addition to selective inhibition of COX-2, which has proven to be problematic. We also discuss cur-*

**Correspondence:** Lenard M. Lichtenberger, Ph.D., Department of Integrative Biology & Pharmacology, The University of Texas Health Science Center at Houston, 6431 Fannin Street, Houston, TX 77030, USA. Tel: +1 713 500 6320; Fax: +1 713 500 7444; E-mail: lenard.m.lichtenberger@uth.tmc.edu

*rent alternative approaches being taken to mitigate the GI side-effects of NSAIDs, including developing combination drugs where NSAIDs are packaged with inhibitors of HCl secretion such as proton pump inhibitors or H<sub>2</sub>-receptor antagonists. We then present the rationale for the development of the PC associated NSAID technology which came out of our observation that the mammalian gastric mucosa has hydrophobic, nonwettable properties that provides a barrier to luminal acid, and the role of phospholipids and specifically phosphatidylcholine (PC) in this barrier property. In the last section we review the development of our current lipid-based PC-NSAID formulations and our encouraging preclinical and clinical observations validating their GI safety and therapeutic efficacy.*

## INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are an important class of drug commonly administered for both acute relief of pain, inflammation and fever and a variety of chronic conditions from arthritis to cardiovascular disease, the latter primarily due to association of aspirin consumption and a lowered incidence of stroke and cardiac events (1-4). Furthermore, there is also a compelling body of evidence that the treatment with NSAIDs (notably aspirin and ibuprofen) is associated with a decreased incidence of a number of cancers, with the strongest evidence relating to the use of aspirin for chemoprevention of colorectal carcinoma (5-9). Similarly there is epidemiological evidence that chronic treatment with aspirin and ibuprofen may be associated with a reduced incidence of a number of neurological diseases including Alzheimer's and Parkinson's disease (10-12). The mechanistic basis for the prophylactic efficacy of NSAIDs in the development and progression of these various contrasting disease states has yet to be elucidated, but may relate to the underlying role of inflammation in these diseases and the generation of pro-inflammatory mediators such as prostaglandin E<sub>2</sub> and its downstream effects on numerous pathways affecting apoptosis and cell proliferation.

However, NSAIDs are known to cause gastrointestinal (GI) toxicity that often leads to ulceration or perforation of the GI mucosal lining, a factor that limits their use. The major concern with the chronic usage of aspirin or other NSAIDs is that 30 to 40% of patients using NSAIDs have a GI intolerance to the drugs and suffer from a spectrum of symptoms ranging from dyspepsia to peptic ulcer disease, the latter which may be associated with life-threatening episodes of hemorrhage (13-17). One

clinical study demonstrated that 30% of chronic NSAID users had at least one gastroduodenal ulcer, as observed via endoscopy (13). Furthermore, a retrospective study restricted to rheumatoid arthritis patients in the U.S. concluded that GI complications as a result of NSAID usage are responsible for 400,000 hospitalizations and 16,000 deaths annually in this patient population (13-17).

To address this issue we have developed novel NSAID products utilizing a lipid based formulation containing soy lecithin enriched in phosphatidylcholine (PC) in a neutral lipid oil matrix which is similar to a self-emulsifying drug delivery system (SEDDS) (18, 19). Phosphatidylcholine is a functional excipient that plays a key role as a solubilizing agent via the formation of a noncovalent complex with the active ingredient NSAID. Evidence supporting the formation of a noncovalent complex is based upon findings that NSAIDs induce profound changes in the biophysical characteristics of PC (e.g., changes to the fluidity and reactivity with hydrophobic fluorescent probes, the transition temperature from gel to liquid crystalline state, its partition coefficient, its solubility in aqueous and organic solvents, and its infrared and vibrational spectra) and vice versa, PC changes a number of the biophysical properties of NSAIDs, as demonstrated by ours and other laboratories (20-27). The observation that the aforementioned changes in biophysical characteristics are reversible, further suggests that the association between NSAIDs and PC is not permanent and therefore, noncovalent in nature. By association with the active ingredient, the PC-NSAID complex becomes markedly more lipophilic. This enhanced lipid solubility of the drug promotes its transit across the hydrophobic mucus gel layer of the upper GI tract, presumably the stomach, with reduced surface mucosal injury. The PC-containing oil excipient neither impedes the bioavailability of the NSAID nor changes the pharmacological activity. The PC lipid based NSAID products currently being developed by Plx Pharma offer lower risk of gastrointestinal erosion and ulceration while maintaining the pharmacological activity and bioavailability demonstrated by the commercial NSAID drug products.

## RATIONALE FOR THE DEVELOPMENT OF PC-NSAIDS

The gastric epithelium is a complex barrier that is designed to withstand the hostile acidic environment of the gastric lumen (2, 28-30). The protective mucus gel layer is predominately composed of mucus glycoproteins, bicarbonate ions and surface active phospholipids.

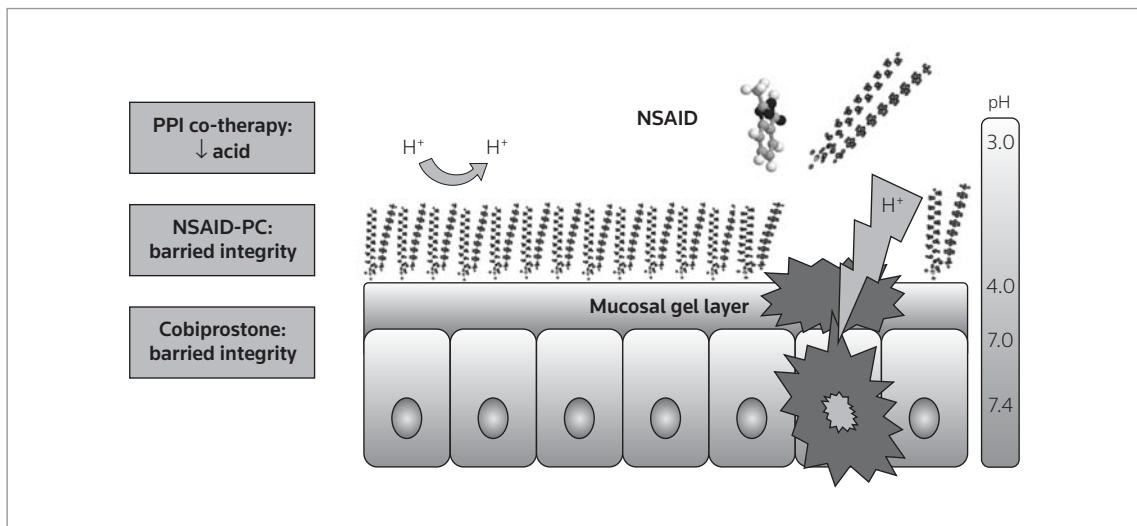
The mucosal surfactants (phospholipids) form a nonwettable, hydrophobic lining that limits the entrance of acid and protects the underlying epithelium (28, 29). Davenport originally suggested that aspirin induced gastric damage was because of injury and alteration of the gastric mucosal barrier resulting in aberrant back diffusion of luminal acid (31). Our technology is based on evidence that injury to the gastric epithelial surface is related to chemical interactions and association of aspirin and other NSAIDs with the mucosal surface active phospholipids, which are secreted by gastric mucus cells into the overlying mucus gel layer (20, 28, 29).

NSAIDs such as aspirin disrupt the natural barrier mechanism of the gastric epithelium, because they bind to the mucosal surfactants. When NSAIDs associate with surface phospholipids the hydrophobic barrier becomes hydrophilic allowing acid to permeate the mucosal lining resulting in disruption of mucosal integrity. As the cells lining the stomach acidify, surface and intracellular membranes break down. With recurrent insult, cells continue to die, resulting in gastric erosions and ulcers. If these NSAID-induced mucosal lesions occur adjacent to an underlying blood vessel, bleeding ulcers can result in potentially life threatening episodes of hemorrhage. Figure 1 depicts our model to explain how the phospholipid extracellular lining at the mucus gel-luminal interface provides a hydrophobic barrier to luminal acid which becomes compromised due to the surface binding

of NSAIDs, such as aspirin, ibuprofen and naproxen, resulting in an increase in acid back-diffusion into the mucosa.

### GASTRIC SURFACE HYDROPHOBICITY: ITS MEASUREMENT AND PERTURBATION BY NSAIDS

Surface active phospholipids in the mucus gel layer are important hydrophobic components of the gastric barrier to acid and consequently binding of NSAIDs to them rapidly attenuates the tissue's surface hydrophobicity (28, 29). The importance of these local effects of aspirin and other NSAIDs has been clinically validated by the ability of antisecretory drugs such as esomeprazole and famotidine to decrease the proton gradient and thus the motive force for acid back diffusion across the mucosa resulting in a decreased risk of gastroduodenal ulceration (20, 28, 29, 32). As an objective benchmark of mucosal barrier integrity, our laboratory measured the hydrophobicity of the gastric luminal surface by evaluating strips of canine gastric mucosa by contact angle analysis (32, 33). Determination of the relative hydrophobicity of a particular surface such as the gastric lining is performed by analysis of the contact angle, which is the angle formed at the air-liquid-solid triple point of the surface and is generally regarded as a reliable index of the hydrophobic characteristics of a particular test surface. A microliter droplet (of fluid such as water) will bead up and form a large contact angle on a



**Figure 1.** A schematic model of the stomach's hydrophobic mucosal barrier and how it is compromised by nonsteroidal anti-inflammatory drugs, promoting the back-diffusion of luminal acid.

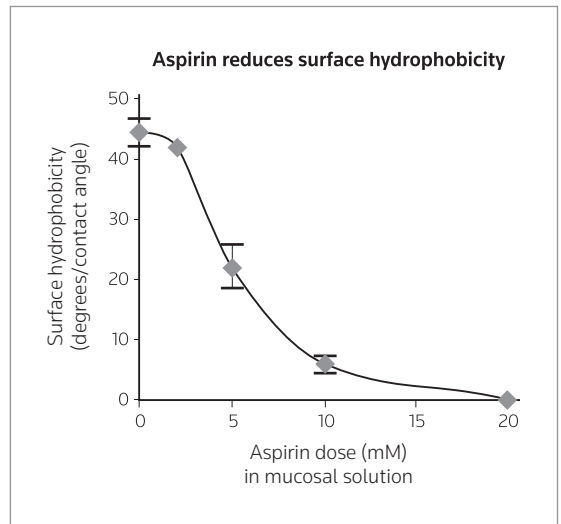
nonwetable hydrophobic surface and conversely, rapidly spread over a hydrophilic interface forming a negligible or small contact angle with that surface. On a perfectly wettable surface the contact angle approaches 0°, whereas on a very hydrophobic surface such as Teflon®, angles of over 100° have been observed (33).

Measurement of gastric surface hydrophobicity has been performed in a number of animal species including humans by placing air-dried mucosal biopsies on the stage of a goniometer (an instrument used to measure contact angles) and carefully applying a small droplet of fluid on the tissue sample. The contact angle for freshly excised tissue was determined to be between 70° and 80°, but equilibrates to approximately 40° to 50° under in vitro conditions, as studies in our Ussing Chamber (a tool to measure ion transport across an epithelium) experiments depicted in Figure 2. These observations show that hydrophobic properties of the gastric mucosa is markedly and dose-dependently decreased after treatment with acidified aspirin (32). The results also suggest that gastric injury may be attenuated by incorporating the NSAID in a lipidic particle, as a complex with PC, to modify interactions with the mucosal membrane and maintain surface hydrophobicity and barrier integrity.

#### CURRENT APPROACHES AND ALTERNATIVES TO MITIGATE NSAID GASTRIC TOXICITY

Because of the health risks associated with chronic NSAID usage, a number of approaches have been utilized to reduce NSAID induced GI toxicity. Combinations of NSAIDs with antisecretory drugs (e.g., H-2 receptor blockers or proton pump inhibitors), cyclooxygenase-2 (COX-2) selective inhibitors (coxibs) and/or the 'cytoprotective' prostaglandin, misoprostol have been developed and marketed. However, due to the limited gastroprotective effect of these commercial products or unexpected adverse events there is a continued effort to develop new strategies to reduce NSAID induced GI toxicity (17, 30). Emerging approaches include the use of NSAIDs in association with nitric oxide, sulfhydryl-containing agents (34-36) and cobiprostone, an eicosanoid known to be a chloride channel activator, currently being developed by Sucampo Pharmaceuticals, Inc. (37-41).

Although the development of coxibs has dominated the approach of pharmaceutical industry over the past 10 years toward NSAID treatment for pain and inflammation, based on the premise that COX-1 inhibition is mostly responsible for NSAID induced GI injury, there is also



**Figure 2.** Ability of aspirin to induce a dose-dependent decrease in gastric surface hydrophobicity of canine gastric mucosa mounted in an Ussing Chamber. Modified with permission from Goddard, P.J., Hills, B.A., Lichtenberger, L.M. *Does aspirin damage canine gastric mucosa by reducing its surface hydrophobicity?* Am J Physiol, 1987, 252(3 Pt 1): G421-30.

much overlooked evidence that NSAID injury to the gut epithelia can be dissociated from the COX inhibitory mechanism altogether. Ligumsky et al demonstrated that despite evidence that intragastrically and parenterally administered aspirin inhibited gastric prostaglandin biosynthesis in a comparable fashion (95%), only the former induced significant gastric injury to the stomachs of rats (42). Darling et al found that aspirin can induce gastric injury in transgenic mice deficient in either COX-1 or COX-2, suggesting that simple inhibition of COX-1 activity cannot provide the only explanation for NSAID induced gastric pathogenesis (43). In addition, there is now compelling evidence that drugs designed to selectively inhibit COX-1 are not damaging to the gut, indicating that the coxib approach may have been based on erroneous presumptions on the importance of this COX isoform, or for that matter prostaglandins, in NSAID induced GI injury (44).

Recent approaches to reduce NSAID induced GI injury relate to the coadministration of the antisecretory drugs with NSAIDs such as esomeprazole and famotidine in an effort to decrease the proton gradient and thus the motive force for acid back diffusion across the mucosa, and thus decreasing the risk of gastroduodenal ulceration (45-47). With a lower motive force, the transit of pro-

tons through NSAID induced mucosal breaks is decreased resulting in lower epithelial cell acidification and injury. Misoprostol, which is used in combination with diclofenac in the drug, Arthrotec® also address the potential GI toxicity of NSAIDs by fortifying the mucosal barrier to acid integrity or turnover by sparing 'cytoprotective' mucosal prostaglandins (48, 49). The major drawback with this approach relates to both its limited GI protective efficacy and the stimulatory effect of the prostanoid on uterine and GI smooth muscle, which may have to potential to cause abortive contractions, and bloating and diarrhea, respectively (49, 50).

Our approach directly addresses the issue of GI toxicity and mucosal lining integrity through application of a specialized lipid based formulation that facilitates safe transit of the NSAID across the mucus gel layer while maintaining the integrity of this barrier to acid and other luminal damaging agents. To decrease NSAID induced surface injury, a novel, oral NSAID drug delivery system has been developed to create a presumptive non-covalent complex between natural (soy) PC and the anti-inflammatory drug. Based upon our laboratory experience the association of the NSAID and PC appears to render the drug more lipophilic and facilitates its transit across the GI mucosa while minimizing surface injury with no loss in functional bioavailability and therapeutic efficacy (20).

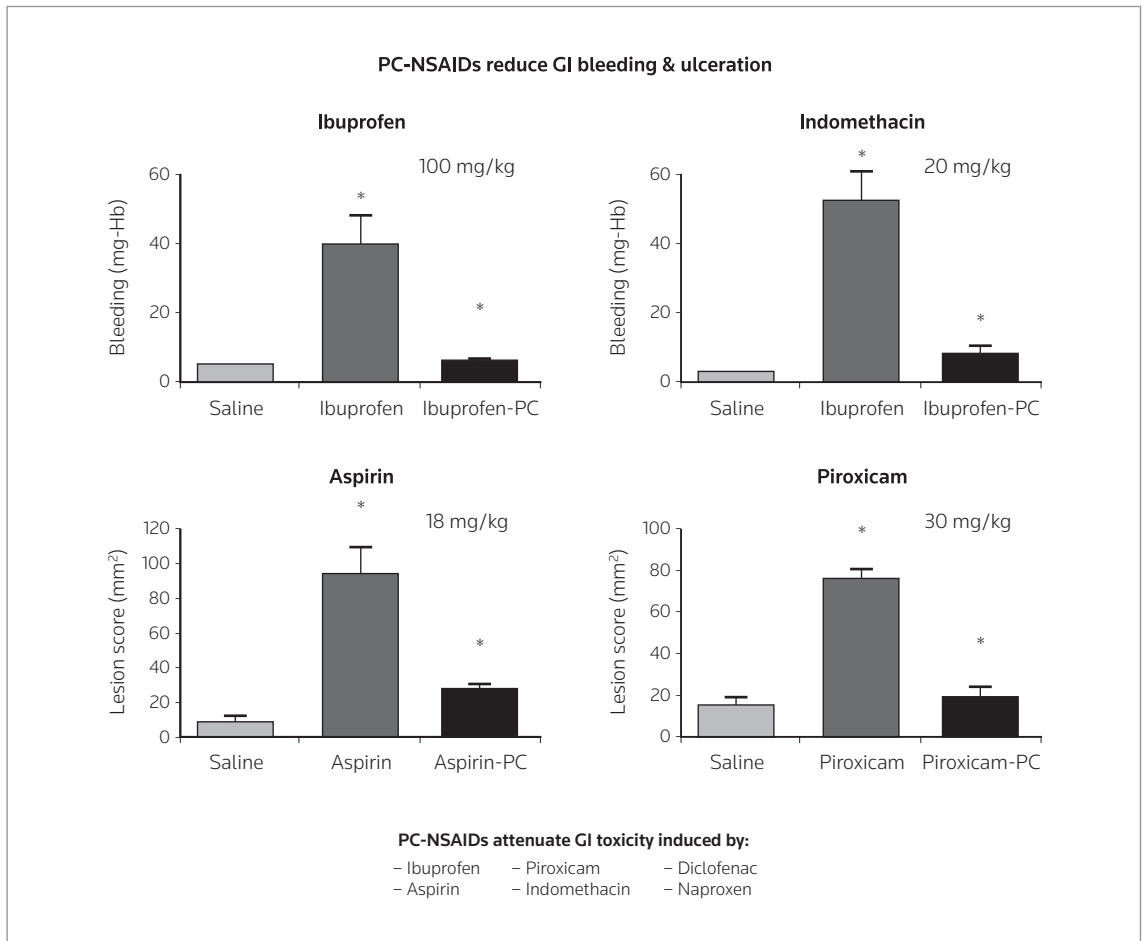
## PHOSPHATIDYLCHOLINE-ASSOCIATED NSAIDS UNDER DEVELOPMENT

### Preclinical evaluation

The development of the PC-associated NSAID technology has undergone an evolution over the past 15 years, since our early studies in the mid 1990s where we demonstrated that a number of NSAIDs could be associated with synthetic (disaturated) dipalmitoyl PC and, when intragastrically administered as an aqueous suspension to rats, reduce NSAID induced gastric injury (Figure 3) (20). Currently with the support of our university-based start-up company, PLx Pharma, Inc. together with grant support from the National Institute of Health and the State of Texas, we have developed a process in which we formulate NSAIDs with a PC-enriched soy lecithin oil together with agents (to reduce viscosity, sedimentation and oxidation and promote dissolution) which is in turn, encapsulated into gelatin capsules. Based upon shelf-life analysis, we have selected the type of capsule to prevent cross-linking and hydrolysis of the active.

PLx Pharma, Inc. is focused on the development of PC conjugates with three widely used NSAIDs, ibuprofen, aspirin and naproxen; and currently has approved investigational new drug (IND) applications for all three. We are currently evaluating the long term shelf life stability of all three PC associated NSAID formulations, which appear to meet expectations necessary for commercialization. Further, the characterization of the structure of the PC associated NSAID lipidic particles within the oil matrix is underway, with much of the evidence supporting the possibility that they exist as reverse micelles. In all cases the optimization of the PC-associated NSAID formulations is based on close collaboration between our research lab and the company, in which prototypes are evaluated in a number of animal models of NSAID induced GI injury, bleeding and therapeutics. In addition to assessing GI injury and bleeding, we also evaluate the efficacy and potency of the test drugs to inhibit inflammation and hyperalgesia in animals where a small volume of complete Freund's adjuvant (CFA) has been injected into their hindpaw to induce inflammation. For our oral formulations, which is the subject of this review, the PC-enriched oil formulations are either dispersed in water and intragastrically administered by gavage or alternatively injected into Torpac Minicaps® which are then intragastrically administered followed by a small volume of water, to more closely simulate human consumption.

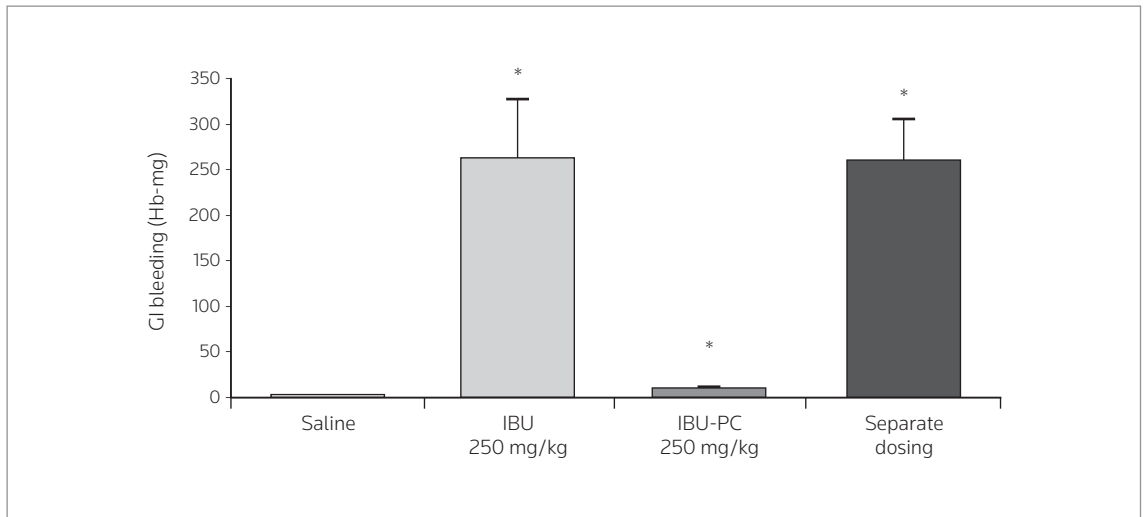
One of the issues investigated was whether or not there is an advantage in pre-associating the NSAID with the PC-enriched oil separately. This question was investigated in an acute GI bleeding model where rats receive injections of the NO-inhibitor, *N*-nitro-L-arginine methyl ester hydrochloride 1 hour before and at 1 and 6 hours after being intragastrically dosed with the test NSAIDs. After 18 hours, the rats are euthanized and the distal half of the small intestine was flushed with saline and the perfusate analyzed for hemoglobin as an index of GI bleeding. In the case of separate dosing, the test NSAID (in this case ibuprofen) was administered either immediately before or after the animals were dosed with the PC-enriched oil and compared to rats that received a single intragastric dose of the ibuprofen pre-associated with PC in the oil matrix. The results demonstrate that in sharp contrast to the impressive reduction in GI bleeding of pre-associated ibuprofen/PC conjugate, separate dosing of ibuprofen and the PC-enriched oil has little or no protective action (Figure 4).



**Figure 3.** Protective efficacy of phosphatidylcholine (PC) against nonsteroidal anti-inflammatory drug (NSAID) induced gastrointestinal (GI) injury using different rodent models of acute NSAID induced injury and bleeding. In this composite figure we present evidence that PC reduces GI injury and bleeding induced by: (A) ibuprofen, (B) indomethacin, (C) aspirin and (D) piroxicam; we also have obtained evidence for the GI protective activity of PC-associated naproxen (as depicted in Figure 7) and diclofenac (not shown). \*In all cases, the PC-associated NSAIDs induced a statistically significant difference vs. the unmodified NSAID ( $P < 0.05$ ); \*\*the unmodified NSAID in each case induced a significant increase in GI lesions and bleeding versus saline treated control; the number of rats/group range between 6 and 15. Modified with permission from Lichtenberger, L.M., Wang, Z.M., Romero, J.J., Ulloa, C., Perez, J.C., Giraud, M.N., Barreto, J.C. *Non-steroidal anti-inflammatory drugs (NSAIDs) associate with zwitterionic phospholipids: insight into the mechanism and reversal of NSAID-induced gastrointestinal injury.* *Nat Med* 1995, 1(2): 154-8.

In a recent rodent study, we compared the ability of PC-associated aspirin with unmodified aspirin to exacerbate gastric injury if administered in combination the coxib, celecoxib (51). This model was originally described by Wallace and associates has the premise that the GI protective action of a coxib is lost if administered together with aspirin (or another COX-1 inhibitor) and has since been confirmed in clinical studies (44, 52). In our study

we investigated the effects of aspirin or the PC-associated aspirin (administered at an NSAID dose of either 20 or 40 mg/kg) alone and in combination with a celecoxib (15 mg/kg) on gastric ulcerogenesis, bleeding, surface hydrophobicity and ulcer healing. Both the effects of PC associated aspirin on the GI tract and COX inhibition levels were evaluated in this study. By measuring both macroscopic gastric lesion formation and changes in



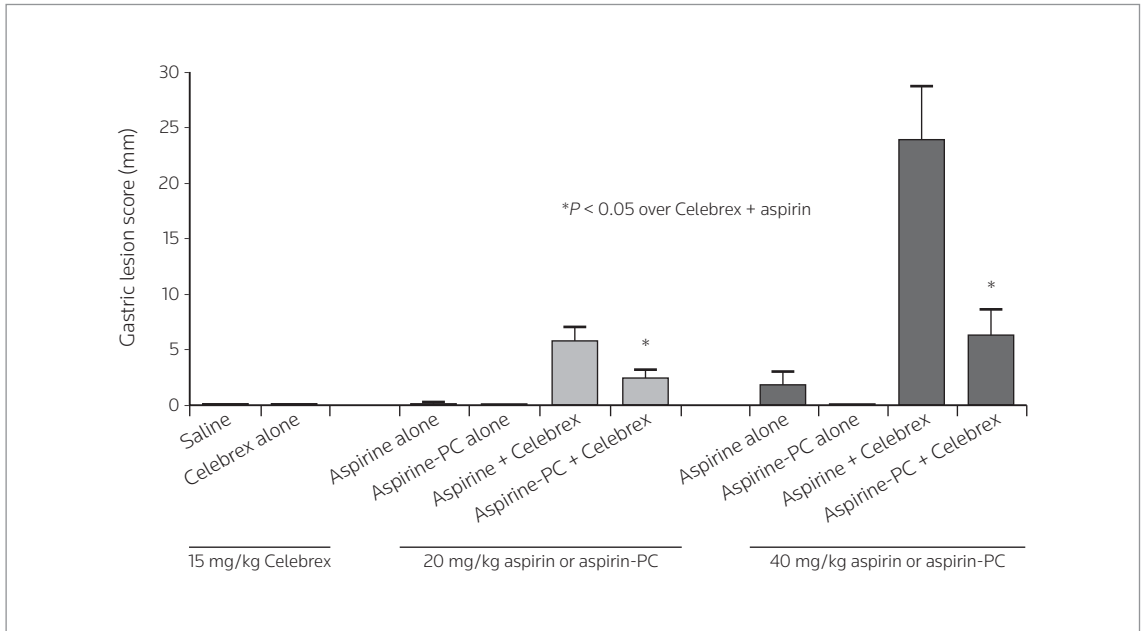
**Figure 4.** Demonstration that pre-association of ibuprofen and phosphatidylcholine (IBU-PC) is required for gastrointestinal protection versus either ibuprofen alone (IBU) or a separate dosing of PC-enriched oil and ibuprofen (Separate dosing). \* $P \leq 0.05$ .

gastric surface hydrophobicity (by contact angle analysis on gastric biopsies), we found that PC-associated aspirin in combination with celecoxib greatly reduced aspirin/celecoxib GI toxicity (Figure 5). In contrast to the ability of aspirin (at a dose of 40 mg/kg) to reduce surface hydrophobicity in the presence and absence of celecoxib, surface hydrophobicity was maintained in rats treated with PC-associated aspirin (Figure 6). Because mucosal surface hydrophobicity is associated with vulnerability to acid induced injury, these data suggest that the tissue's ability to resist the corrosive effects of acid is maintained after PC-associated treatment.

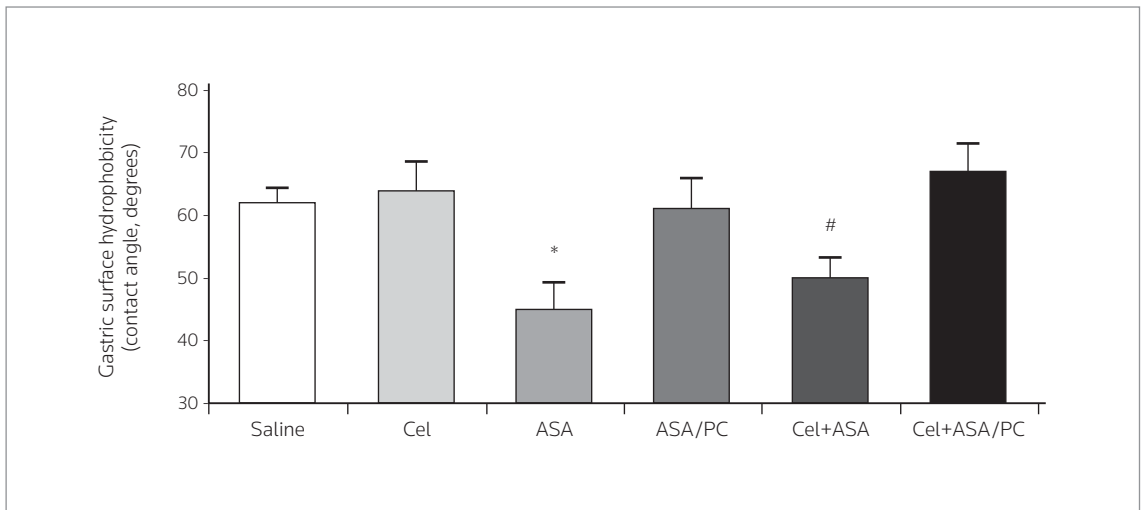
In the same study we demonstrated that experimentally induced gastric ulcers healed better when rats were administered with celecoxib in combination with PC-associated aspirin (with results similar to the saline control) than with the celecoxib in combination with unmodified aspirin. We also demonstrated that PC-associated aspirin and aspirin had a comparable inhibitory effect on mucosal prostaglandin  $E_2$  concentration, indicating that the protective effect of PC-conjugated aspirin is independent of COX inhibition.

Another model system routinely used to evaluate the safety and efficacy of the PC-NSAID formulation was used in a recent paper in the journal *Inflammopharmacology* where both the GI safety and therapeutic efficacy of a PC-associated naproxen formulation were evaluated (53). In this series of experiments, the hind

paw of rats was injected with 0.2 mL CFA and then the animals were intragastrically administered naproxen or PC-associated naproxen (25 mg/kg bid) or the equivalent volume of saline for 3 days, during which time fecal matter was collected to assess fecal hemoglobin concentration, as an index of GI bleeding. At the end of the study period the rats were euthanized and the following parameters assessed: the thickness of the rat's paw was measured to assess edema and synovial fluid was collected to measure the COX-mediated generation of the prostaglandin  $E_2$ ; blood was collected to assess hematocrit (an indicator of anemia induced by chronic GI bleeding); and the small intestine was dissected and macroscopically inspected for the presence of perforations and adhesions. The results depicted in Figure 7A-D provide evidence that PC-associated naproxen induced significantly less GI injury and bleeding than an equivalent dose of unmodified naproxen, while inducing a significant inhibition in ankle edema and synovial fluid prostaglandin  $E_2$  as indices of the formulation's anti-inflammatory and COX-inhibitory efficacy, respectively. It should be noted, that although the COX-inhibitory activity of PC-associated naproxen and unmodified naproxen were comparable in this study, it appeared that ankle edema in the PC-associated-naproxen-treated group was less than that observed in the group treated with unmodified naproxen. If reproducible these findings with PC-associated naproxen would run counter to earlier observations where we reported in different rodent



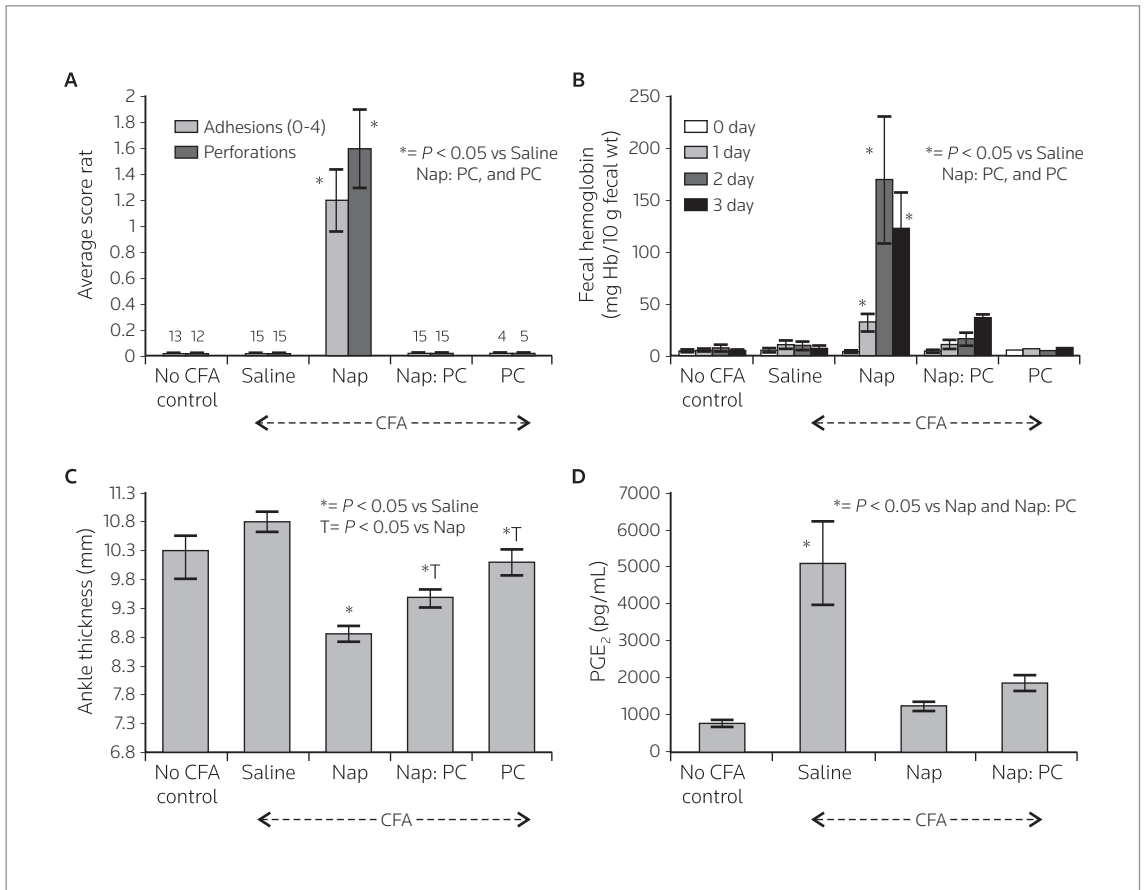
**Figure 5.** Gastroprotective efficacy of aspirin-phosphatidylcholine (PC) vs. aspirin alone and in combination with celecoxib. Phosphatidylcholine associated aspirin in combination with celecoxib reduced gastric lesion score when compared to aspirin alone in combination with celecoxib. \* $P < 0.05$ . Modified with permission Lichtenberger, L.M., Romero, J.J., Dial, E.J. *Surface phospholipids in gastric injury and protection when a selective cyclooxygenase-2 inhibitor (Coxib) is used in combination with aspirin.* Br J Pharmacol 2007, 150(7): 913-9.



**Figure 6.** Gastric surface hydrophobicity is maintained in rats treated with phosphatidylcholine (PC) associated aspirin when compared to an equivalent dose of aspirin (40 mg/kg) either alone or in combination with a celecoxib. Cel = celecoxib, ASA = aspirin, ASA/PC = PC-associated aspirin. Modified with permission Lichtenberger, L.M., Romero, J.J., Dial, E.J. *Surface phospholipids in gastric injury and protection when a selective cyclooxygenase-2 inhibitor (Coxib) is used in combination with aspirin.* Br J Pharmacol 2007, 150(7): 913-9.

model systems that PC-associated ibuprofen had significantly greater analgesic and anti-inflammatory activity than observed with equivalent (non-ulcerogenic) doses of the respective unmodified NSAID (54). It should be noted that in the subchronic experiments depicted in Figure 7, where an ulcerogenic dose of naproxen was used, the analgesic efficacy of test formulations could not be assessed due to the fact that the animals have visceral injury and pain that would confound this behavioral analysis.

In order to assess the analgesic efficacy of our test formulations, we use a modification of the above model system. First, animals with CFA induced joint inflammation are fasted overnight on the 3rd day after CFA injection. Second, the following day after a baseline measurement of the pain pressure threshold (to be described below) the animals are acutely treated with a single intragastric dose of PC associated ibuprofen, unmodified ibuprofen or saline. Third, 2 hours later, the sensitivity of the animal's inflamed hind paw to pressure was evaluated using our modification of the established Randall



**Figure 7.** Subchronic model used to evaluate the GI safety (A and B) and therapeutic efficacy (C and D) of phosphatidylcholine (PC)-associated naproxen versus unmodified naproxen in rats with complete Freund's adjuvant-induced joint inflammation by measuring: (A) intestinal adhesions and perforations ( $P < 0.05$  when comparing naproxen alone to saline, PC-associated naproxen or PC alone); (B) fecal hemoglobin ( $P < 0.05$  when comparing naproxen alone to saline, PC-associated naproxen or PC alone); (C) ankle thickness ( $P < 0.05$  when comparing naproxen alone to saline, PC-associated naproxen or PC alone); and (D) synovial fluid prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) concentration ( $P < 0.05$  when comparing saline to PC-associated naproxen or naproxen alone). The numbers above bars = number of rats/group. Modified with permission from Lichtenberger, L.M., Romero, J.J., Dial, E.J., Moore, J.E. *Naproxen-PC: A GI safe and highly effective anti-inflammatory*. *Inflammopharmacology* 2008, 16(1): 1-5.

**Table I.** The pharmacokinetics of phosphatidylcholine-associated ibuprofen after initial dose and repeat administration over 4 weeks in evaluable patients.

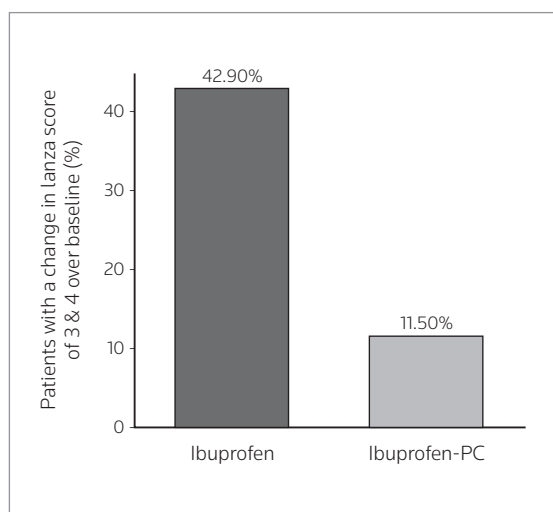
Treatment	PK-parameter	Day 0		Week 4		Raw change		Paired t-test	t-test
		N	Mean ± STD	N	Mean ± STD	N	Mean ± STD	P value	P value
Ibuprofen	C <sub>max</sub> (mg/L)	64	53.0 ± 20.0	59	58.6 ± 19.3	59	5.1 ± 23.5	0.1005	0.5725
Ibuprofen-PC	C <sub>max</sub> (mg/L)	61	62.5 ± 24.8	54	71.0 ± 21.1	54	7.8 ± 27.3	0.0399*	0.5725
Ibuprofen	T <sub>max</sub> (min)	64	80.6 ± 27.5	59	81.7 ± 28.0	59	1.7 ± 29.6	0.6680	0.6713
Ibuprofen-PC	T <sub>max</sub> (min)	61	76.7 ± 27.6	54	76.3 ± 25.1	54	-0.9 ± 33.2	0.8511	0.5725

Ibuprofen-PC, phosphatidylcholine-associated ibuprofen; PK, pharmacokinetic; STD, standard deviation. With permission from Lanza, F.L., Marathi, U.K., Anad, B.S., Lichtenberger, L.M. *Clinical trial: Comparison of ibuprofen-phosphatidylcholine and ibuprofen on the gastrointestinal safety and analgesic efficacy in osteoarthritic patients.* *Aliment Pharmacol Ther* 2008, 28(4): 431-42.

Selitto procedure (54). The method requires using an analgesymeter which applies increasing pressure on the paw until the animal first senses pain (termed the 'pain pressure threshold') as indicated by paw withdrawal or extension of the digits. Using this model system, we have confirmed that the analgesic efficacy of PC-associated ibuprofen to increase the pain pressure threshold over baseline is comparable with that of the unmodified ibuprofen and significantly greater than that recorded in saline treated controls, thereby demonstrating analgesic efficacy of PC associated ibuprofen (54).

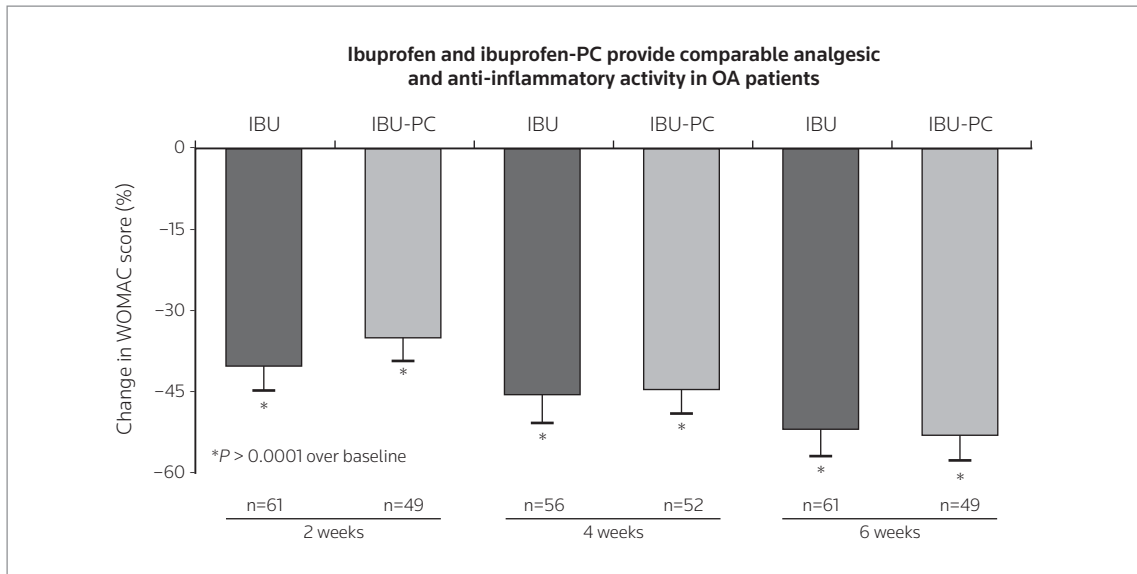
### Clinical Evaluation

Currently PLx Pharma, Inc. has had IND applications approved by the United States Food and Drug Administration for PC-associated ibuprofen (PL-1100; PL-1200), PC-associated aspirin (PL-2200) and PC-associated naproxen (PL-3100) and all three oil matrix PC-associated NSAID formulations are in different phases of clinical evaluation of their pharmacokinetics, safety and GI toxicity. In a paper published in the journal *Alimentary Pharmacology & Therapeutics* (55), we reported that a PC-associated ibuprofen formulation had a pharmacokinetic drug profile bioequivalent to unmodified ibuprofen in osteoarthritic patients at a dose of 800 mg TID, and that the PC-associated ibuprofen formulation induced significantly fewer gastroduodenal clinically relevant endoscopic ulcers and erosions than ibuprofen alone ( $P < 0.015$ ) after a 6-week dosing period in patients older than 55 years of age, without any treatment related adverse events (Table I and Figure 8). We have also demonstrated in the same study that PC-associated ibuprofen provided relief of pain and inflammation in osteoarthritic patients during the trial period that was comparable with ibuprofen using both a Western



**Figure 8.** Phosphatidylcholine (PC)-associated ibuprofen induced clinically relevant gastroduodenal endoscopic injury (using Lanza scoring system where Lanza score of  $> 2 = 3-10$  erosions or an ulcer) in a significantly ( $P < 0.015$ ) lower percentage (11.5%) of treated at-risk osteoarthritic (OA) subjects, older than 55 years of age, than was observed with unmodified ibuprofen (42.9%) when the drugs were administered at a dose of 2,400 mg ibuprofen/day for 6 weeks. Modified with permission from Lanza, F.L., Marathi, U.K., Anand, B.S., Lichtenberger, L.M. *Clinical trial: Comparison of ibuprofen-phosphatidylcholine and ibuprofen on the gastrointestinal safety and analgesic efficacy in osteoarthritic patients.* *Aliment Pharmacol Ther* 2008, 28(4): 431-42.

Ontario & McMaster universities arthritis criteria index and VAS-patient global assessment scoring systems (see Figure 9). Previously we demonstrated in cross-over single dose pharmacokinetic (Phase I/II) trials in healthy subjects that PC associated ibuprofen was bioequiva-



**Figure 9.** Both phosphatidylcholine (PC)-associated ibuprofen (IBU-PC) and ibuprofen (IBU) statistically significantly reduced (when compared to baseline) the Western Ontario & McMaster universities arthritis criteria index (WOMAC) score a comparable extent in osteoarthritis subjects over the 6-week study period, indicating that therapeutic efficacy of the test drugs to inhibit pain and inflammation was comparable. \* $P < 0.0001$  over baseline. Modified with permission from Lanza, F.L., Marathi, U.K., Anand, B.S., Lichtenberger, L.M. *Clinical trial: Comparison of ibuprofen-phosphatidylcholine and ibuprofen on the gastrointestinal safety and analgesic efficacy in osteoarthritic patients.* *Aliment Pharmacol Ther* 2008, 28(4): 431-42.

lence to unmodified ibuprofen, when the two drugs were evaluated at doses of 200, 400 and 800 mg. Taking a similar approach we have recently completed clinical pharmacokinetic, bioequivalent and a 7-day GI safety endoscopic assessment of PC-associated aspirin compared to aspirin in healthy subjects and have initiated pharmacokinetic evaluation of PC-associated naproxen.

## CONCLUSIONS

The PC-associated NSAID products being developed directly address the issue of GI toxicity typically associated with NSAID therapies. An NSAID pre-associated with PC in a soy lecithin oil matrix is administered as a liquid-filled gelatin capsule. The key lipid in the formulation, PC, interacts and forms a (non-covalent) complex with the NSAID producing a molecular dispersion with enhanced lipid solubility. By formulating the NSAID as a complex with PC the direct association of the potentially toxic drug with native mucosal phospholipids in the GI mucus gel layer is prevented and GI injury is significantly reduced or eliminated. In addition, upon oral administration the lipid based formulation hydrates and pro-

duces a SEDDS. Due to the large surface area, the SEDDS widely distributes the lipid associated drug and efficiently presents the NSAID to the gastrointestinal tract in a readily transferable state.

Both in vitro and in vivo studies demonstrate that attenuation in gastric hydrophobic barrier is associated with the interaction of NSAIDs with the gastric mucosa through binding to the endogenous PC present within and coating the mucus gel layer. Studies on laboratory animals further support the conclusion that associating NSAIDs with PC reduces the NSAID associated toxicity without affecting efficacy and that NSAID toxicity is associated with the ability of the drug to modify the GI surface hydrophobic barrier through interaction with endogenous PC.

Clinical pharmacology and upper GI endoscopic trials have provided evidence supporting the working hypothesis that an NSAID in the PC-containing oils is predominantly emulsified in the upper GI tract and is delivered across the hydrophobic gastric barrier (to acid) with minimal mucosal injury. Our finding that the pharmacokinetic profiles of our PC-associated NSAIDs appear simi-

lar to unmodified NSAIDs, supports the concept that the PC containing oils act as a drug delivery system that facilitates the transit of the NSAID across the gastric mucosal gel layer without disrupting the protective barrier. The PC excipient neither impedes the bioavailability of the NSAID nor adds any pharmacological activity. Thus this new class of PC associated NSAIDs appears to offer lower risk of GI erosion and ulceration while maintaining the pharmacological activity and bioavailability demonstrated by commercial NSAID drug products.

## DISCLOSURES

Part of this work was supported by: NIH grants R42 DK 063882, P30DK 056338, and R44 ARO56529, the State of Texas from an Emerging Technology Fund grant; and PLx Pharma, Inc. Dr Lichtenberger has served as an officer, director, consultant, owns stock and has received research funding from PLx Pharma, Inc., Dr. Marathi is an employee and owns stock in PLx Pharma, Inc. and Dr. Barron is a contractor for PLx Pharma, Inc.

## REFERENCES

1. Furst, D.E., Paulus, H.E. *Aspirin and other nonsteroidal anti-inflammatory drugs*. In: *Arthritis and allied conditions*, McCarty D.J., Koopman, W.J. (Eds). Lea & Febiger, Philadelphia, PA, USA Twelfth Edition 1993, 567-602.
2. Wallace, J.L. *Nonsteroidal anti-inflammatory drugs and gastroenteropathy: The second hundred years*. *Gastroenterology* 1997, 112(3): 1000-16.
3. Pelletier, J-P. *Pathological pathways of osteoarthritis* In: *Non-steroidal anti-inflammatory drugs: A research and clinical perspective*. Royal Society of Medicine Press, London, UK 1994, 1-14.
4. Hebert, P.R., Hennekens, C.H. *An overview of the 4 randomized trials of aspirin therapy in the primary prevention of vascular disease*. *Arch Intern Med* 2000, 160(20): 3123-7.
5. Cook, N.R., Lee, I.M., Gaziano, D. et al. *Low-dose aspirin in the primary prevention of cancer: The Women's Health Study: a randomized controlled trial*. *JAMA* 2005, 294(1): 47-55.
6. Chan, A.T., Giovannucci, E.L., Meyerhardt, J.A., Schernhammer, E.S., Wu, K., Fuchs, C.S. *Aspirin dose and duration of use and risk of colorectal cancer in men*. *Gastroenterology* 2008, 134(1): 21-8.
7. Flossmann, E., Rothwell, P.M., British Doctors Aspirin Trial and the UK-TIA Aspirin Trial. *Effect of aspirin on long-term risk of colorectal cancer: Consistent evidence from randomised and observational studies*. *Lancet* 2007, 369(9573): 1603-13.
8. Cole, B.R., Logan, R.F., Halabi, S. et al. *Aspirin for the chemoprevention of colorectal adenomas: Meta-analysis of the randomized trials*. *J Natl Cancer Inst* 2009, 101: 256-66.
9. Chan, A.T., Ogino, S., Fuchs, C.S. *Aspirin use and survival after diagnosis of colorectal cancer*. *JAMA* 2009, 302(6): 649-58.
10. in t' Veld, B.A., Ruitenbergh, A., Hofman, A. et al. *Nonsteroidal anti-inflammatory drugs and the risk of Alzheimer's disease*. *N Engl J Med* 2001, 345: 1515-21.
11. Etminan, M., Gill, S., Samii, A. *Effect of non-steroidal anti-inflammatory drugs on risk of Alzheimer's disease: Systematic review and meta-analysis of observational studies*. *BMJ* 2003, 327(7407): 128.
12. Samii, A., Etminan, M., Wiens, M.O., Jafari, S. *NSAID use and the risk of Parkinson's disease: systematic review and meta-analysis of observational studies*. *Drugs Aging* 2009, 26(9): 769-79.
13. Gabriel, S.E., Jaakkimainen, L., Bombardier, C. *Risk for serious gastrointestinal complications related to use of nonsteroidal anti-inflammatory drugs. A meta-analysis*. *Ann Intern Med* 1991, 115(10): 787-96.
14. Allison, M.C., Howatson, A.G., Torrance, C.J., Lee, F.D., Russell, R.I. *Gastrointestinal damage associated with the use of nonsteroidal anti-inflammatory drugs*. *N Engl J Med* 1992, 327(11): 749-54.
15. Hawkey, C.J. *Nonsteroidal anti-inflammatory drug gastropathy*. *Gastroenterology* 2000, 119(2): 521-35.
16. Wolfe, M.M., Lichtenstein, D.R., Singh, G. *Gastrointestinal toxicity of nonsteroidal anti-inflammatory drugs*. *N Engl J Med* 1999, 340(24): 1888-99.
17. Lanas, A., García-Rodríguez, L.A., Arroyo, M.T. et al. *Risk of upper gastrointestinal ulcer bleeding associated with selective cyclo-oxygenase-2 inhibitors, traditional non-aspirin non-steroidal anti-inflammatory drugs, aspirin and combinations*. *Gut* 2006, 55(12): 1731-8.
18. Porter, C.J., Trevaskis, N.L., Charman, W.N. *Lipids and lipid-based formulations: Optimizing the oral delivery of lipophilic drugs*. *Nat Rev Drug Discov* 2007, 6(3): 231-48.
19. Gursoy, R.N., Benita, S. *Self-emulsifying drug delivery systems (SEDDS) for improved oral delivery of lipophilic drugs: drug delivery and drug efficacy*. *Biomed Pharmacother* 2004, 58(3): 173-82.
20. Lichtenberger, L.M., Wang, Z.M., Romero, J.J., Ulloa, C., Perez, J.C., Giraud, M.N., Barreto, J.C. *Non-steroidal anti-inflammatory drugs (NSAIDs) associate with zwitterionic phospholipids: Insight into the mechanism and reversal of NSAID-induced gastrointestinal injury*. *Nat Med* 1995, 1(2): 154-8.
21. Lichtenberger, L.M., Wang, Z.M., Giraud, M.N., Romero, J.J., Barreto, J.C., *Effect of naproxen (NAP) on gastric mucosal hydrophobicity: Possible interaction with surface phospholipids*. *Gastroenterology*, 1995. 108(4 Suppl 1): A149.
22. Giraud, M.N., Motta, C., Romero, J.J., Lichtenberger, L.M. *Effect of indomethacin (Indo) on the physical state of gastric surface-active phospholipids (PL)*. *Gastroenterology* 1996, 110: A116.

23. Ferreira, H., Lucio, M., de Castro, B., Gamiro, P., Lima, J.L.F.C., Reis, S. *Partition and location of nimesulide in EPC liposomes: A spectrophotometric and fluorescence study.* Anal Bioanal Chem 2003, 377(2): 293-8.
24. Kyrikou, I., Hadjikakou, S.K., Kovala-Demertzi, D., Viras, K., Mavromoustakos, T. *Effects of non-steroid anti-inflammatory drugs in membrane bilayers.* Chem Phys Lipids 2004, 132(2): 157-69.
25. Lichtenberger, L.M., Dial, E.J., Jayaraman, V. et al. *Evaluation of the structural properties and activities of a purified PC-NSAID preparation.* Gastroenterology 2006, 130: A270.
26. Lúcio, M., Bringezu, F., Reis, S., Lima, J.L., Brezesinski, G. *Binding of nonsteroidal anti-inflammatory drugs to DPPC: structure and thermodynamic aspects.* Langmuir 2008, 24(8): 4132-9.
27. Moreno, M.M., Garidel, P., Suwalsky, M., Howe, J., Brandenburg, K. *The membrane-activity of ibuprofen, diclofenac, and naproxen: A physico-chemical study with lecithin phospholipids.* Biochim Biophys Acta 2009, 1788(6): 1296-303.
28. Lichtenberger, L.M., Zhou, Y., Dial, E.J., Raphael, R.M. *NSAID injury to the gastrointestinal tract: evidence that NSAIDs interact with phospholipids to weaken the hydrophobic surface barrier and induce the formation of unstable pores in membranes.* J Pharm Pharmacol 2006, 58(11): 1421-8.
29. Lichtenberger, L.M. *The hydrophobic barrier properties of gastrointestinal mucus.* Annu Rev Physiol 1995, 57: 565-83.
30. Laine, L., Takeuchi, K., Tarnawski, A. *Gastric mucosal defense and cytoprotection: Bench to bedside.* Gastroenterology 2008, 135(1): 41-60.
31. Davenport, H.W. *Damage to the gastric mucosa: Effects of salicylates and stimulation.* Gastroenterology 1965, 49: 189-96.
32. Goddard, P.J., Hills, B.A., Lichtenberger, L.M. *Does aspirin damage canine gastric mucosa by reducing its surface hydrophobicity?* Am J Physiol, 1987, 252(3 Pt 1): G421-30.
33. Hills, B.A., Butler, B.D., Lichtenberger, L.M. *Gastric mucosal barrier: Hydrophobic lining to the lumen of the stomach.* Am J Physiol 1983, 244(5): G561-8.
34. Wallace, J.L., Calendo, G., Santiagada, V., Cirino, G., Forucci, S. *Gastrointestinal safety and anti-inflammatory effects of a hydrogen sulfide-release diclofenac derivative in the rat.* Gastroenterology 2007, 132(1): 261-71.
35. Wallace, J.L. *Building a better aspirin: Gaseous solutions to a century old problem.* Br J Pharmacol 2007, 152(4): 421-8.
36. Davies, N.M., Røseth A.G., Appleyard, C.B. et al. *NO-naproxen vs. naproxen: Ulcerogenic, analgesic and anti-inflammatory effects.* Aliment Pharmacol Ther 1997, 11(1): 69-79.
37. Cuppoletti, J.; Malinowska, D.H.; Chakrabarti, J.; Ueno, R. *Cobiprostone protects against indomethacin damage through activation of Clc-2: A gene chip analysis and Clc-2 ablation study.* Dig Dis Week (May 30-June 4, Chicago) 2009, Abst S1766.
38. Osama, H.; Kuno, S.; Roerig, B.; Ueno, R. *Effect of cobiprostone on naproxen-induced gastric ulcers in rats.* Gastroenterology 2008, 134(4, Suppl. 1): Abst W1939.
39. Osama, H.; Kuno, S.; Roerig, B.; Ueno, R. *Effects of cobiprostone on indomethacin- and stress-induced gastric ulcers in rats.* Am J Gastroenterol 2007, 102(Suppl. 2): Abst 115
40. Cuppoletti, J.; Mende, K.; Malinowska, D.H.; Ueno, R. *Cobiprostone is a type-2 chloride channel activator that protects against NSAID-induced cellular damage.* Am J Gastroenterol 2007, 102(Suppl. 2): Abst 126.
41. *Cobiprostone Prevention of NSAID-Induced Gastroduodenal Injury (NCT00597818).* ClinicalTrials.gov Web site, December 1, 2009.
42. Ligumsky, M., Grossman, M.I., Kauffman, G.L. Jr. *Endogenous gastric mucosal prostaglandins: Their role in mucosal integrity.* Am J Physiol 1982, 242(4): G337-41.
43. Darling, R.L., Romero, J.J., Dial, E.J., Akunda, J.K., Langenbach, R., Lichtenberger, L.M. *The effects of aspirin on gastric mucosal integrity, surface hydrophobicity, and prostaglandin metabolism in cyclooxygenase knockout mice.* Gastroenterology 2004, 127(1): 94-104.
44. Wallace, J.L., McKnight, W., Reuter, B.K., Vergnolle, N. *NSAID-induced gastric damage in rats: Requirement for inhibition of both cyclooxygenase 1 and 2.* Gastroenterology 2000, 119(3): 706-14.
45. Scheiman, J.M., Yeomans, N.D., Talley, N.J. et al. *Prevention of ulcers by esomeprazole in at-risk patients using non-selective NSAIDs and COX-2 inhibitors.* Am J Gastroenterol 2006, 101(4): 701-10.
46. Yeomans, N., Lanasa, A., Labenz, J. et al. *Efficacy of esomeprazole (20 mg once daily) for reducing the risk of gastroduodenal ulcers associated with continuous use of low-dose aspirin.* Am J Gastroenterol 2008, 103(10): 2465-73.
47. Taha, A.S., McCloskey, C., Prasad, R., Bezlyak, V. *Famotidine for the prevention of peptic ulcers and oesophagitis in patients taking low-dose aspirin (FAMOUS): A phase III, randomised, double-blind, placebo-controlled trial.* Lancet 2009, 374(9684): 119-25.
48. Dajani, E.S., Agrawal, N.M. *Prevention and treatment of ulcers induced by nonsteroidal anti-inflammatory drugs: An update.* J Physiol Pharmacol 1995, 46(1): 3-16.
49. Morant, S.V., Shield, M.J., Davey, P.G., MacDonald, T.M. *A pharmacoeconomic comparison of misoprostol/diclofenac with diclofenac.* Pharmacoeconomic Drug Saf 2002, 11(5): 393-400.
50. Winer, N., Resche-Rigon, M., Morin, C., Ville, Y., Rozenburg, P. *Is induced abortion with misoprostol a risk factor for late abortion or preterm delivery in subsequent pregnancies?* Eur J Obstet Gynecol Reprod Biol 2009, 145(1): 53-6.

51. Lichtenberger, L.M., Romero, J.J., Dial, E.J. *Surface phospholipids in gastric injury and protection when a selective cyclooxygenase-2 inhibitor (Coxib) is used in combination with aspirin.* Br J Pharmacol 2007, 150(7): 913-9.
52. Wallace, J.L., Zamuner, S.R., McKnight, W., Dickey, M., Mencarelli, A., del Soldato, P., Fiorucci, S. *Aspirin, but not NO-releasing aspirin (NCX-4016), interacts with selective COX-2 inhibitors to aggravate gastric damage and inflammation.* Am J Physiol Gastrointest Liver Physiol 2004, 286(1): G76-81.
53. Lichtenberger, L.M., Romero, J.J., Dial, E.J., Moore, J.E. *Naproxen-PC: A GI safe and highly effective anti-inflammatory.* Inflammopharmacology 2008, 16(1): 1-5.
54. Lichtenberger, L.M., Romero, J.J., de Ruijter, W.M., Behbod, F., Darling, R., Ashraf, A.Q., Sanduja, S.K. *Phosphatidylcholine association increases the anti-inflammatory and analgesic activity of ibuprofen in acute and chronic rodent models of joint inflammation: Relationship to alterations in bioavailability and cyclooxygenase-inhibitory potency.* J Pharmacol Exp Ther 2001, 298(1): 279-87.
55. Lanza, F.L., Marathi, U.K., Anand, B.S., Lichtenberger, L.M. *Clinical trial: Comparison of ibuprofen-phosphatidylcholine and ibuprofen on the gastrointestinal safety and analgesic efficacy in osteoarthritic patients.* Aliment Pharmacol Ther 2008, 28(4): 431-42.
- .....