

Research Article

Naproxen-PC: A GI safe and highly effective anti-inflammatory

L. M. Lichtenberger^{1*}, J. J. Romero², E. J. Dial¹ and J. E. Moore²

¹Department of Integrative Biology & Pharmacology, The University of Texas Health Science Center at Houston, Houston, TX, USA,
Fax: 1-(713) 500-7444, e-mail: Lenard.M.Lichtenberger@uth.tmc.edu

²PLx Pharma Inc, Houston, TX, USA

Received 13 October 2008; accepted 11 November 2008

Abstract. We have been developing a family of phosphatidylcholine (PC)-associated NSAIDs, which appear to have improved GI safety and therapeutic efficacy in both rodent model systems and pilot clinical trials. As naproxen has been demonstrated to be associated with the lowest cardiovascular adverse events in comparison with both COX-2 selective inhibitors and conventional NSAIDs, we have been developing a Naproxen-PC formulation for evaluation in animal models and clinical trials. We have determined that an oil-based formulation of naproxen and triple strength soy lecithin provides excellent GI protection in both: 1) an acute NSAID-induced intestinal bleeding model in rats pretreated with L-NAME that are intragastrically administered a single dose of naproxen (at a dose of 50 mg/kg) vs the equivalent dose of Naproxen-PC; and 2) a more chronic model (at a naproxen dose of 25 mg/kg BID) in rats that have pre-existing hindpaw inflammation (induced with a intradermal injection of Complete Freund's Adjuvant/CFA). Both models demonstrate the superior GI safety of Naproxen-PC vs naproxen while this novel formulation had significant anti-inflammatory efficacy to reduce hindpaw edema and the generation of PGE₂ in the collected joint synovial fluid. Conclusion: Naproxen-PC appears to induce significantly less GI injury and bleeding in two rodent model systems while maintaining anti-inflammatory and COX-inhibitory activity.

Key words: Naproxen – Phosphatidylcholine – Ulcer – Inflammation – Cyclooxygenase – Prostaglandin.

Introduction

One of the central mechanisms by which nonsteroidal anti-inflammatory drugs (NSAIDs) induce GI injury and bleeding is by disruption of the tissue surface barrier to gastric acid and

other luminal cytotoxic agents (Hills et al., 1983, Goddard et al., 1990, Goddard et al., 1987, Lichtenberger, 1995, Giraud et al., 1999). Our lab has provided insight into the molecular basis of this barrier property by demonstrating that the mucus gel layer of the stomach and other regions of the GI tract possess non-wettable hydrophobic properties due to the synthesis and secretion of surfactant-like phospholipids, with phosphatidylcholine (PC) being the most prominent (Butler et al., 1983, Hills et al., 1983, Lichtenberger et al., 1983, Lichtenberger, 1995, Lichtenberger et al., 2007). We have also demonstrated that this surface hydrophobic barrier can be rapidly attenuated by NSAIDs and other damaging agents and/or conditions (Lichtenberger, 1995, Goddard et al., 1990, Giraud et al., 1999). We are still exploring the molecular basis for this action, but have evidence that NSAIDs chemically interact with and associate with PC, thereby disrupting its surface barrier properties. Based upon this understanding we have determined that pre-association of a number of NSAIDs with synthetic or natural (soy) PC can provide significant protection against NSAID-induced GI injury and bleeding in both rodent model systems and pilot clinical trials (Giovannucci et al., 1995, Anand et al., 1999, Lichtenberger, 1995, Lichtenberger et al., 2007).

Our approach of maintaining the surface barrier of the GI tract is quite different from the conventional approach taken by the pharmaceutical industry over the past decade in the development of COX-2 selective inhibitors (coxibs), based upon the concept that non-specific NSAIDs induce GI injury primarily by inhibiting constitutive COX-1, and thereby depleting the tissue of “cytoprotective” prostaglandins (Jüni et al., 2002, Insel, 1996, Budenholzer, 2002). Although endoscopic clinical trials and outcome studies appeared to confirm that a number of coxibs were indeed, safer to the GI tract than conventional NSAIDs in arthritic patients chronically taking the drugs at high dose (Cronstein, 2002, Deeks et al., 2002), new evidence also was revealed that coxibs and certain NSAIDs increased a patient's risk of developing serious life-threatening cardiovascular adverse

* Corresponding author

events including: unstable angina; myocardial infarction; heart failure; and ischaemic stroke. This concern was further corroborated in subsequent studies in both arthritic patients and patients taking lower chemo-preventive doses of these drugs leading to the withdrawal of most coxibs from the market and “black box” warnings placed on all NSAIDs (Bresalier et al., 2005, Solomon et al., 2005, Nussmeier et al., 2005, Zarraga and Schwarz, 2007). In these studies it was concluded that the NSAID that presented the least cardiovascular risk and providing the most effective relief for arthritic patients is naproxen (Watson et al., 2002, Farkouh et al., 2007). However since naproxen, especially at arthritic doses, is significantly toxic to the GI tract (Biskupiak et al., 2006, Chan, 2006, Garcia Rodriguez and Jick, 1994), there remains a great unmet need for a GI safe-formulation of this NSAID. It is, therefore, our goal to develop and evaluate a Naproxen-PC formulation using our rodent model systems that can ultimately be translated into the clinic.

Materials and methods

Animal Experiments

We used two animal models of NSAID-induced GI injury. The acute model strictly provides a rapid screening tool to assess and compare the GI toxicity of a single dose of the test NSAIDs, as determined by an increase in intestinal bleeding 20–24 hrs after dosing. The subchronic model allows one to assess the test NSAID's: 1) GI toxicity, with regards to both GI bleeding (faecal haemoglobin, haematocrit reduction), the development of intestinal perforation and adhesions; 2) therapeutic anti-inflammatory efficacy, with regards to inhibiting COX activity and edema of the CFA-injected hindpaw; and 3) overall effect on the health of the animals, with regards to gain in body weight over the 3-day dosing period. The acute and subchronic rodent model systems that were used to evaluate the GI safety and therapeutic effectiveness of our test naproxen formulations are described below.

L-NAME acute ulcer model: The acute intestinal toxicity of the test agents was evaluated in L-NAME (N-nitro-L-arginine methyl ester hydrochloride, L-NAME, 20 mg/kg, i.p.)-treated rats that were intragastrically administered saline, Nap (40 mg/kg) or an equivalent dose of Nap-PC as previously outlined (Lichtenberger et al., 2001), and 18 hrs later the rats were euthanized and the distal half of the small intestine flushed with saline and analyzed for hemoglobin (Hb) as an estimate of GI bleeding as previously described.

CFA-induced joint inflammation model: Complete Freund's Adjuvant (CFA) was injected (0.2 ml) into a hindpaw of rats, followed by intragastric administration (25 mg/kg, oral, BID for 3 days) of either naproxen (Nap), naproxen that was associated with phosphatidylcholine (Nap-PC), or saline. In this model system, Complete Freund's Adjuvant (CFA) is injected subcutaneously into a rat's hind paw to induce joint inflammation. Test NSAIDs can be administered daily during the 3-day study period by one of several different routes. The advantage of this model is that it can provide data on the test NSAIDs' anti-inflammatory efficacy, as well as their GI side effects (ulceration and bleeding), all from the same animal. The anti-inflammatory effects can be assessed by measuring the joint swelling by caliper, and the generation of eicosanoid mediators of inflammation by RIA in the synovial fluid as previously described (Anand et al., 1999; Lichtenberger et al., 2001; Darling et al., 2004). Lastly, GI side-effects (ulceration/bleeding) can be assessed by daily measurement of faecal haemoglobin and at euthanasia by measuring haematocrit (index of anaemia caused by GI bleeding), and inspecting the GI tract for lesions, perforations and adhesions. Intestinal adhesions were assessed by a scale of 0 to 4. The small intestines were removed as a mass and were gently pulled apart for assessment of sticking and scored as follows: 0 = normal; 1 = slight sticking, pull apart without tearing; 2 = sticking, must cut adhesions to pull apart; 3

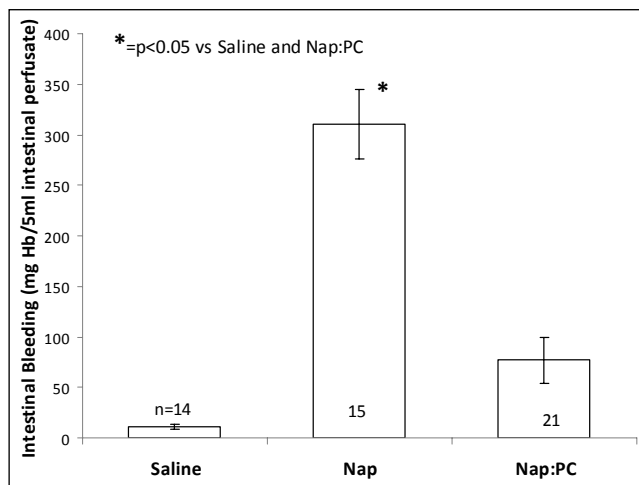


Fig. 1. Demonstration that Naproxen-PC induces significantly less GI bleeding than an equivalent dose of 40 mg Naproxen/kg, using the acute L-NAME rodent model system.

= numerous adhesions, difficulty in cutting intestines apart; 4 = massive adhesions, cannot be cut apart. Intestinal perforations were counted after filling the distal half of the small intestine with 5 ml of water and counting the number of breaks where leaks occurred.

PGE₂ analysis: The PGE₂ in synovial fluid from rat paws was assayed directly by radioimmunoassay using antibody from Sigma Chemical Co. (St. Louis, MO) and the manufacturer's instructions.

Statistical analysis: Experiments were analyzed initially by one-way ANOVA, followed by post hoc testing with the Fisher LSD test. The level of significance was set at $p < 0.05$.

Results

Acute NSAID-induced GI bleeding

We evaluated the acute GI protective action of Naproxen-PC vs naproxen in rats, using the L-NAME model system. Briefly syntheses rats, that have been dosed with the constitutive NO-inhibitor to increase their sensitivity to the GI toxic actions of NSAIDs, are intragastrically administered a single dose of the test NSAIDs or saline, and 18 hrs later the distal half of the small intestine is flushed and the haemoglobin (Hb) concentration of the perfusate analyzed to assess NSAID-induced GI bleeding. The results depicted in Figure 1 indicate that naproxen induced a significant ten-fold increase in GI bleeding vs the values of saline-treated controls. Most importantly, Naproxen-PC induced a highly significant 75–80% reduction in GI bleeding in comparison to rats that were administered an equivalent dose of unmodified naproxen.

Subchronic NSAID-induced efficacy & GI injury in rats with CFA-induced joint inflammation

Based upon the very encouraging results outlined above, we next used a second model which provides information on both the GI toxicity and anti-inflammatory efficacy of the

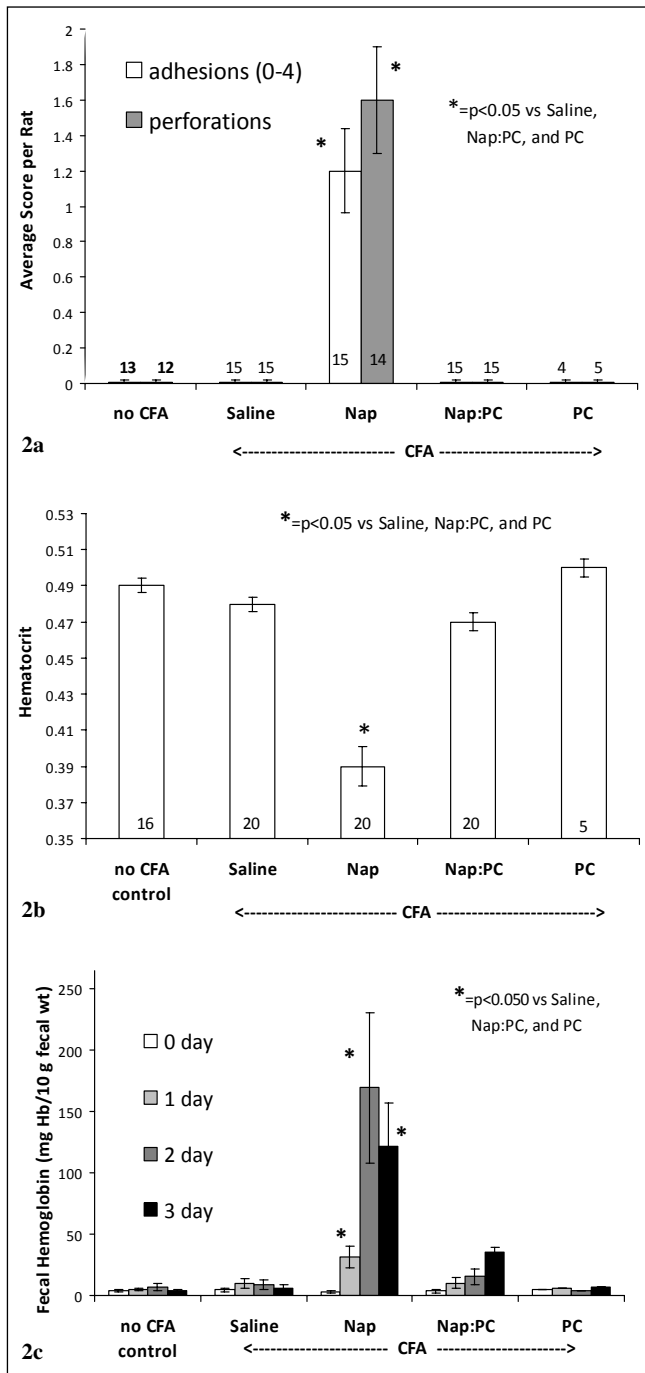


Fig. 2. Evaluation of the GI toxicity of Naproxen-PC in comparison to an equivalent dose of naproxen (25 mg/kg, bid for 3 days) using a subchronic rodent model of CFA-induced joint inflammation. The superior GI-safety of Naproxen-PC vs naproxen is evident based on: (A) macroscopic assessment of perforations/adhesions; (B) haematocrit determination, with a reduction in this value serving as an estimate of the development of an anaemia, presumably caused by NSAID-induced GI bleeding; and (C) analysis of faecal haemoglobin over the 3-day study period.

test NSAIDs (or vehicle), which were intragastrically administered twice daily to rats with CFA-induced joint inflammation. Figures 2 & 3 represent such an experiment where we compared the anti-inflammatory and GI side-effects of intra-

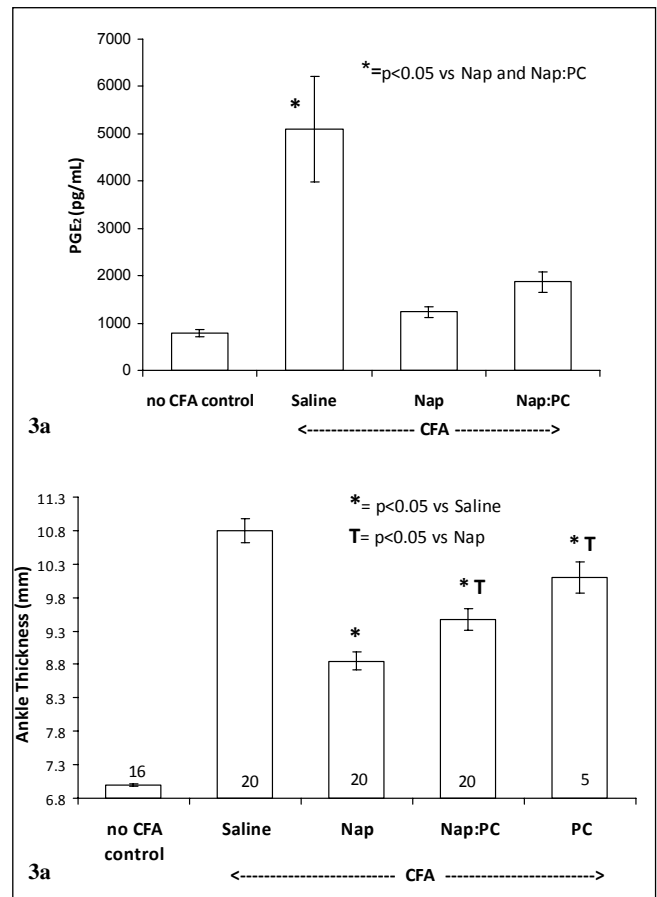


Fig. 3. Evaluation of the therapeutic efficacy of Naproxen-PC in comparison to an equivalent dose of naproxen (25 mg/kg, bid for 3 days) using a subchronic rodent model of CFA-induced joint inflammation, as determined by measuring: (A) PGE₂ concentration of the synovial fluid as an estimate of the COX-inhibitory activity of test NSAID-formulations; and (B) the ankle thickness of the CFA-affected hindpaw at the end of the 3-day study period.

gastric naproxen (25 mg/kg, BID), to an equivalent (NSAID) dose and/or volume of Naproxen-PC or saline. Figure 2 A–C demonstrates that in this subchronic model, Naproxen-PC is significantly less GI toxic than naproxen, inducing markedly fewer intestinal perforations and adhesions (A) and a decrease in GI bleeding, as reflected by both a reduction in haematocrit (B) as an index of anaemia, and decrease in faecal Hb (C) over the 3-day study period.

Figure 3 demonstrates that Naproxen-PC possesses significant anti-inflammatory activity, as indicated by a decrease in ankle thickness in the CFA-injected hindpaw, as well as COX-inhibitory activity, as indicated by a decrease in PGE₂ concentration of the synovial fluid collected from the affected joint. It should be noted that although Naproxen-PC had comparable COX-inhibitory activity to naproxen, inhibiting PGE₂ concentration by >85% from control (saline dosed) CFA-affected joint fluid (see Fig 3A), it was somewhat less effective in reducing joint edema in comparison to the unmodified NSAID (Fig 3B). It also can be appreciated that PC on its own was GI-safe and demonstrated no detectable anti-inflammatory or COX-inhibitory activity.

Discussion

In this study we have evaluated the GI safety and therapeutic efficacy of a novel formulation of naproxen, in which the NSAID is formulated with soy PC, promoting the chemical (non-covalent) association of these two classes of molecules. This approach was taken based upon previous pre-clinical studies and pilot clinical trials indicating that Aspirin-PC and Ibuprofen-PC possess lower GI toxicity and equivalent therapeutic efficacy to the parent drug (Lichtenberger et al., 1995, Lanza et al., 2008). In the current study we used two rodent models to demonstrate the superior GI safety of Naproxen-PC vs naproxen after either a single or multiple doses of the test NSAIDs, based upon several indices of GI bleeding (intestinal and faecal Hb, and haematocrit determination as an estimate of the development of anaemia), as well as the macroscopic appearance of intestinal perforations and adhesions in the subchronic model. It also appeared that Naproxen-PC possessed significant anti-inflammatory and COX-inhibitory activity, based upon the reduction in ankle thickness and synovial PGE₂ concentration in the hindpaw of rats exposed to CFA and subsequently treated intragastrically for 3 days with the test NSAIDs or vehicle.

Future studies are planned to further evaluate how the therapeutic (anti-inflammatory and analgesic) efficacy of Naproxen-PC compares with unmodified naproxen. The data presented above suggest that although the COX inhibitory activity may have been greater for Naproxen-PC than naproxen, it may have been less effective to reduce paw inflammation. It should be noted that we opted against measuring the analgesic efficacy of the test formulations (by measuring the sensitivity of the CFA-affected hindpaw to either thermal or mechanical stimulation) in the subchronic model system, as the results would have been confounded by NSAID-induced visceral pain, which presumably would have been reduced in the rats treated with our novel NSAID formulation. It however should be noted that in a recent clinical trial it was determined that the therapeutic efficacy of another PC-NSAID, Ibuprofen-PC, to inhibit pain/inflammation in patients with osteoarthritis was comparable to that of the parent NSAID, ibuprofen, based upon inducing a significant change, comparing baseline to post-treatment using standardized (WOMAC and VAS) scoring protocols, over the 6-week study period (Lanza et al., 2008).

As previously discussed, there is a great unmet need for the development of an NSAID that can effectively treat chronic pain and inflammation without also inducing major cardiovascular and GI adverse events. Recent evidence suggests that, although quite GI toxic, naproxen appears to be the safest NSAID for induction of cardiac events, thrombosis and/or hypertension (Chan, 2006). We present here pre-clinical evidence that pre-association of naproxen with PC in an oil-based soy lecithin formulation effectively reduces the GI toxicity of this NSAID, while appearing to maintain its therapeutic effects, which likely is due to its profound COX-inhibitory activity. Future pre-clinical and clinical trials are planned to both confirm these findings and to translate them clinically.

References

- Anand, B.S., Romero, J.J., Sanduja, S.K. & Lichtenberger, L.M. (1999) Phospholipid association reduces the gastric mucosal toxicity of aspirin in human subjects. *Am J Gastroenterol*, **94**, 1818–22.
- Biskupiak, J.E., Brixner, D.I., Howard, K. & Oderda, G.M. (2006) Gastrointestinal complications of over-the-counter nonsteroidal anti-inflammatory drugs. *J Pain Palliat Care Pharmacother*, **20**, 7–14.
- Bresalier, R.S., Sandler, R.S., Quan, H., Bolognese, J.A., Oxenius, B., Horgan, K., Lines, C., Riddell, R., Morton, D., Lanasa, A., Konstam, M.A., & Baron, J.A. (2005) Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial. *N Engl J Med*, **352**, 1092–102.
- Budenholzer, B.R. (2002) Are selective COX 2 inhibitors superior to traditional NSAIDs? Rofecoxib did not provide unequivocal benefit over traditional NSAIDs. *BMJ*, **325**, **161**; author reply **161**.
- Butler, B.D., Lichtenberger, L.M., & Hills, B. A. (1983) Distribution of surfactants in the canine gastrointestinal tract and their ability to lubricate. *Am J Physiol*, **244**, G645–51.
- Chan, F. K. (2006) Primer: managing NSAID-induced ulcer complications—balancing gastrointestinal and cardiovascular risks. *Nat Clin Pract Gastroenterol Hepatol*, **3**, 563–73.
- Cronstein, B. N. (2002) Cyclooxygenase-2-selective inhibitors: translating pharmacology into clinical utility. *Cleve Clin J Med*, **69 Suppl 1**, S113–9.
- Darling, R. L., Romero, J. J., Dial, E. J., Akunda, J. K., Langenbach, R. & Lichtenberger, L.M. (2004) The effects of aspirin on gastric mucosal integrity, surface hydrophobicity, and prostaglandin metabolism in cyclooxygenase knockout mice. *Gastroenterology*, **127**, 94–104.
- Deeks, J. J., Smith, L. A. & Bradley, M. D. (2002) Efficacy, tolerability, and upper gastrointestinal safety of celecoxib for treatment of osteoarthritis and rheumatoid arthritis: systematic review of randomised controlled trials. *BMJ*, **325**, 619.
- Farkouh, M. E., Greenberg, J. D., Jeger, R. V., Ramanathan, K., Verheugt, F. W., Chesebro, J. H., Kirshner, H., Hochman, J. S., Lay, C. L., Ruland, S., Mellein, B., Matchaba, P. T., Fuster, V. & Abramson, S. B. (2007) Cardiovascular outcomes in high risk patients with osteoarthritis treated with ibuprofen, naproxen or lumiracoxib. *Ann Rheum Dis*, **66**, 764–70.
- Garcia Rodriguez, L. A. & Jick, H. (1994) Risk of upper gastrointestinal bleeding and perforation associated with individual non-steroidal anti-inflammatory drugs. *Lancet*, **343**, 769–72.
- Gionannucci, E., Egan, K. M., Hunter, D. J., Stampfer, M. J., Colditz, G. A., Willett, W. C. & Speizer, F. E. (1995) Aspirin and the risk of colorectal cancer in women. *N Engl J Med*, **333**, 609–14.
- Giraud, M. N., Motta, C., Romero, J. J., Bommalaer, G. & Lichtenberger, L.M. (1999) Interaction of indomethacin and naproxen with gastric surface-active phospholipids: a possible mechanism for the gastric toxicity of nonsteroidal anti-inflammatory drugs (NSAIDs). *Biochem Pharmacol*, **57**, 247–54.
- Goddard, P. J., Hills, B. A. & Lichtenberger, L.M. (1987) Does aspirin damage canine gastric mucosa by reducing its surface hydrophobicity? *Am J Physiol*, **252**, G421–30.
- Goddard, P. J., Kao, Y. C. & Lichtenberger, L.M. (1990) Luminal surface hydrophobicity of canine gastric mucosa is dependent on a surface mucous gel. *Gastroenterology*, **98**, 361–70.
- Hills, B. A., Butler, B. D. & Lichtenberger, L.M. (1983) Gastric mucosal barrier: hydrophobic lining to the lumen of the stomach. *Am J Physiol*, **244**, G561–8.
- Insel, P. A. (1996) Analgesic-Antipyretic and antiinflammatory agents and drugs employed in the treatment of gout. In Hardman, J. G. (Ed.) Goodman & Gilman's The Pharmacological Basis of Therapeutics. 9th ed. New York, McGraw-Hill.
- Jüni, P., Rutjes, A. W. & Dieppe, P. A. (2002) Are selective COX 2 inhibitors superior to traditional non steroidal anti-inflammatory drugs? *BMJ*, **324**, 1287–8.
- Lanza, F. L., Marathi, U., Anand, B. S. & Lichtenberger, L.M. (2008) Clinical trial: comparison of ibuprofen-phosphatidylcholine and ibuprofen on the gastrointestinal safety and analgesic efficacy in osteoarthritic patients. *Aliment Pharmacol Ther*, **28**, 431–442.

- Lichtenberger, L.M. (1995) The hydrophobic barrier properties of gastrointestinal mucus. *Annu Rev Physiol*, **57**, 565–83.
- Lichtenberger, L.M., Graziani, L. A., Dial, E. J., Butler, B. D. & Hills, B. A. (1983) Role of surface-active phospholipids in gastric cytoprotection. *Science*, **219**, 1327–9.
- Lichtenberger, L.M., Romero, J. J., De Ruijter, W. M., Behbod, F., Darling, R., Ashraf, A. Q. & Sanduja, S. K. (2001) 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*, **298**, 279–87.
- Lichtenberger, L.M., Romero, J. J. & Dial, E. J. (2007) Surface phospholipids in gastric injury and protection when a selective cyclooxygenase-2 inhibitor (Coxib) is used in combination with aspirin. *Br J Pharmacol*, **150**, 913–9.
- Lichtenberger, L.M., Wang, Z. M., Romero, J. J., Ulloa, C., Perez, J. C., Giraud, M. N. & Barreto, J. C. (1995) Non-steroidal anti-inflammatory drugs (NSAIDs) associate with zwitterionic phospholipids: insight into the mechanism and reversal of NSAID-induced gastrointestinal injury. *Nat Med*, **1**, 154–8.
- Nussmeier, N. A., Whelton, A. A., Brown, M. T., Langford, R. M., Hoelt, A., Parlow, J. L., Boyce, S. W. & Verburg, K. M. (2005) Complications of the COX-2 inhibitors parecoxib and valdecoxib after cardiac surgery. *N Engl J Med*, **352**, 1081–91.
- Solomon, S. D., McMurray, J. J., Pfeffer, M. A., Wittes, J., Fowler, R., Finn, P., Anderson, W. F., Sauber, A., Hawk, E. & Bertagnoli, M. (2005) Cardiovascular risk associated with celecoxib in a clinical trial for colorectal adenoma prevention. *N Engl J Med*, **352**, 1071–80.
- Watson, D. J., Rhodes, T., Cai, B. & Guess, H. A. (2002) Lower risk of thromboembolic cardiovascular events with naproxen among patients with rheumatoid arthritis. *Arch Intern Med*, **162**, 1105–10.
- Zarraga, I. G. & Schwarz, E. R. (2007) Coxibs and heart disease: what we have learned and what else we need to know. *J Am Coll Cardiol*, **49**, 1–14.

To access this journal online:
<http://www.birkhauser.ch/IPh>
