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Development of the PC-NSAID technology: From contact angle to Vazalore®

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We describe strategies in drug development to reduce the gastrointestinal (GI) toxicity of nonsteroidal anti-inflammatory drugs (NSAIDs). We then provide an overview of the experiments that led to the development of PC-NSAIDs, a novel family of NSAIDs associated with phosphatidylcholine (PC) that have reduced GI toxicity and full therapeutic activity. Furthermore, we describe the evidence showing: that the stomach possesses hydrophobic properties that are attributable to phospholipids lining the mucus gel layer; and that NSAIDs chemically associate with intrinsic PC, thereby attenuating the tissue's hydrophobic properties. Further, pre-associating NSAIDs with PC reduces the GI toxicity of these drugs, both in rodent ulcer models and in human subjects, without affecting the drugs' therapeutic activity. Finally, we discuss the commercialization and launch of Aspirin-PC, an over-the-counter (OTC) drug with the brand name Vazalore®.

Keywords: nonsteroidal anti-inflammatory drugs; aspirin; ibuprofen; phospholipids; phosphatidylcholine; hydrophobicity; ulcer; Platelet; inflammation; cyclo-oxygenase; COX

Nonsteroidal anti-inflammatory drugs (NSAID) are a family of pharmaceuticals that possess powerful therapeutic actions in inhibiting inflammation, fever, and pain. Aspirin, the acetylated form of salicylate, was the first NSAID synthesized and remains one of the most consumed pharmaceuticals today.[1] In addition, the consumption of aspirin is associated with reduced incidence of cardiovascular disease, stroke and numerous forms of cancer.[2–4] Pioneering work by the Vane lab, carried out 50 years ago, demonstrated that NSAIDs share the ability to inhibit the generation of prostaglandins via cyclooxygenase (COX) inhibition. Furthermore, this work demonstrated that prostaglandins and related eicosanoids are mediators of inflammation and of platelet aggregation, an important event in blood clotting after injury.[5]

One of the major side effects that limits the consumption of NSAIDs relates to their ability to induce gastrointestinal (GI) issues that include dyspepsia, as well as gastroduodenal erosions and ulcers that can result in life-threatening episodes of GI bleeding or hemorrhage. It has been estimated that 20–40% of NSAID consumers will develop one of these GI symptoms within their lifetime.[6] The mechanism of NSAID injury falls into one of two major categories: (i) COX-dependent mechanisms related to loss of prostaglandins, which possess the ability to protect the gastroduodenal mucosa

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against lumen-damaging agents, labelled as 'cytoprotection' by Robert[7]; (ii) COX-independent actions, which include topical injury to the gastroduodenal mucosa (which is the focus of the phosphatidylcholine (PC)-NSAID technology)[8]; and (iii) intracellular injury, which includes alkalization of the cell (as a result of the protonophore actions of aspirin and related NSAIDs) and disruption or uncoupling of mitochondrial oxidative metabolism.[1,9].

Strategies in the development of drugs or medicines to reduce the GI toxicity of NSAIDs

Enteric-coated aspirin

Relying on the premise that the GI side effects of aspirin primarily occur in the stomach, pharmaceutical companies manufactured aspirin tablets that were coated with silicone or cellulose, thereby delaying the absorption of the drug until it had left the stomach and transited into the small intestine. Although enteric-coating appeared to reduce the gastric irritancy of the drug, early reports indicated that it did not prevent dyspepsia or GI bleeding, which originated in the small bowel.[10] This was subsequently confirmed in a retrospective analysis of six larger studies involving >15,000 subjects, which concluded that enteric coating did not prevent most aspirin-induced GI side effects, including significant GI bleeding that was mostly attributable to GI erosions and ulcers in the upper small bowel.[11]

Combination of NSAIDs with inhibitors of gastric acid secretion

Based on the adage 'no acid no ulcer', one of the strategies taken by both researchers and the pharmaceutical industry was to combine NSAIDs with anti-secretory drugs: either H-2 antagonists (famotidine) or proton pump inhibitors (esomprazole). The novel medicines that were developed as a result of this effort include Duexis®, which combines famotidine with ibuprofen, and Vimovo®, which combines esomprazole with naproxen.[12,13] Clinical trials of these medicines indicated that these drug combinations provided moderate and comparable protection against NSAID-induced gastric injury, but also carry the limitation of compelling consumers who are long-term users of NSAIDs to take these powerful anti-secretory drugs chronically. When proton pump inhibitors (PPIs) are used in these drug combinations, users are placed at increased risk of being susceptible to infections (i.e. *Clostridium difficile*)[14] and osteoporosis.[15]

Gaseous-releasing NSAIDs

An alternative approach to reducing the GI toxicity is to associate an NSAID covalently with a gaseous molecule (NO and/or H_2S) that promotes GI mucosal integrity and, at the same time, promotes the anti-inflammatory action of the drug. This approach was led by the Wallace lab and was the focus of a number of start-up pharmaceutical companies. It resulted in the development of NO- and H_2S-NSAIDs that show promise on the basis of animal testing and pilot clinical trials.[16,17] The most promising candidates are H_2S-naproxen (ATB-346) and H_2S-ketoprofen (ATB-352), which are currently being evaluated in ongoing clinical and pre-clinical trials, respectively, sponsored by Antibe Therapeutics. Finally, a novel class of NSAIDs have been developed that release both NO and H_2S; these NOSH-NSAIDs can inhibit cancer cell growth in laboratory experiments but, to our knowledge, have yet to be evaluated in clinical trials.[16–18]

Phospho-NSAIDs

A family of NSAIDs that are linked by an ester group to a number of phosphate moieties has also been developed.[19] This novel drug class has been reported to have reduced GI toxicity and to possess anti-inflammatory and anti-cancer activities in animal model systems. To our knowledge, this family of NSAIDs has not been evaluated clinically.

Prostaglandins

The prostanoid drug Misoprostol® was developed on the basis of the observation by Robert and associates[7] that prostaglandins have 'cytoprotective' activity. Misoprostol® provides significant protection against NSAID-induced gastroduodenal injury.[20] Subsequently, Arthrotec®, which combines diclofenac and Misoprostol, was developed.[21] Although these prostaglandin-containing medicines protect against NSAID-induced gastroduodenal ulcers, they also have potentially serious side effects, including the induction of smooth muscle contraction of the uterine muscles (causing miscarriages or abortions in pregnant women)[22] or GI contractions (resulting in episodes of diarrhea).[23]

COX-2 inhibitors

Expression of the COX-2 isoform, which was discovered in the 1990s, appears to be associated with inflammation, injury and dysplasia, leading to the development of COX-2 selective inhibitors (coxibs).[24] Coxibs target the form of the enzyme that generates prostaglandins from non-GI tissues subjected to inflammation, injury or dysplasia, but do not affect the GI tissues that constitutively express COX-1, which generates prostaglandins with 'cytoprotective' activity.[24] These observations resulted in the development and commercialization of the highly selective COX-2 inhibitor Vioxx® and the less selective COX-2 inhibitor Celebrex®. Although both coxibs appeared efficacious in treating patients who suffer from chronic inflammation with a reduction in GI ulcers and bleeding,[25,26] subsequent studies indicated that coxibs were unexpectedly associated with potentially serious cardiovascular events,[27] including myocardial infarction, thrombosis and stroke. As a result, Vioxx was withdrawn from the market in 2006, and Blackbox warnings were given to Celebrex and other NSAIDs that are less selective COX-2 inhibitors (i.e. Meloxicam).

Genesis of PC-NSAID technology

Hydrophobic properties of the stomach surface indicated by contact angle analysis

In 1982, our laboratory made the initial observation that the mammalian gastric mucosa has non-wettable hydrophobic properties, as determined by surface wettability analysis using a goniometer.[28] Interestingly, the goniometer reading, which was made by placing a microliter droplet of water on the semi-dry mucosal surface of the body of the stomach, had contact angle readings of >80° at the air/liquid/-solid triple point, approaching the properties of non-wettable surfaces such as Teflon (see Fig. 1). Subsequent laboratory experiments demonstrated that, in comparison, regions of the small bowel had significantly lower contact angle readings (≤10°), which suggest that this is a more wettable surface than the gastric mucosa, as one would expect of a highly absorptive

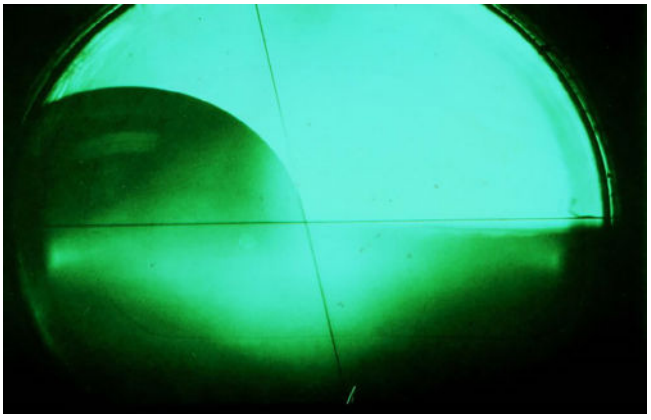

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FIGURE 1

A microliter droplet of water on the surface of mammalian gastric mucosa, forming a prominent 75–80° contact angle at the air/liquid/solid interface. The upper GI tract has hydrophobic surface properties that appear to be associated with an extracellular layer of phospholipids. This surface hydrophobic lining can be rapidly reduced by NSAIDs.

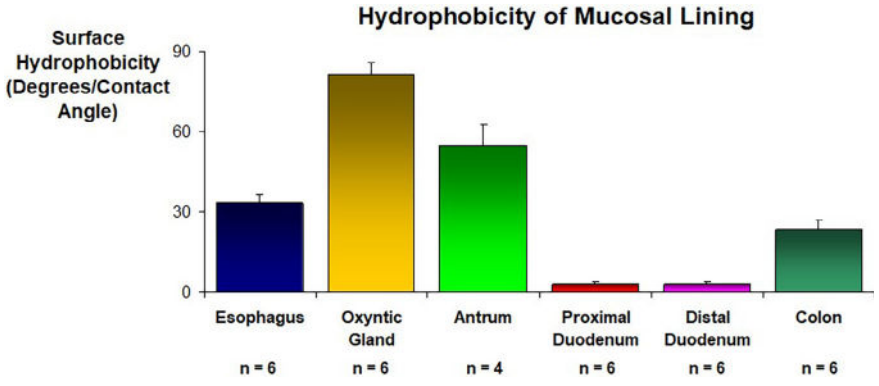

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FIGURE 2

Mucosal surface hydrophobicity along the gastrointestinal (GI) tract. Contact angle readings for canine gastric mucosa from mucosal biopsies along the GI tract. The data indicate that the gastric mucosa is the most hydrophobic part of the GI tract, whereas and the small intestinal mucosa (proximal and distal duodenum) has the lowest readings as might be expected of a hydrophilic surface that promotes the absorption of fluids and non-lipidic nutrients. Redrawn from Hills *et al.*[28]

epithelium (see Fig. 2). In addition to these animal studies, two independent groups of clinical investigators reported that human gastric mucosa also possesses significant hydrophobic properties, as assessed by contact angle analysis of endoscopically collected gastric biopsies.[29,30] Interestingly, this non-wettable surface property was significantly lower in ulcer patients infected with *Helicobacter pylori* but returned to normal levels after the bacterial infection was eradicated.[29,30]

Biochemical and morphological evidence of surface phospholipid lining

Contact angle analyses were followed by biochemical analysis of the apical gastric mucosal surface. This work demonstrated that this outer layer was enriched in phospholipids, with the zwitterionic phospholipid phosphatidylcholine (PC) constituting >40% of the total phospholipids present, and with desaturated dipalmitoyl PC (DPPC) being the most prominent species.[31,32] At this point, we postulated that PC and related zwitterionic phospholipids form a monolayer on the mucosal surface with the hydrophobic fatty acid side chains extending into the lumen, thereby creating a non-wettable surface (see Fig. 3). This possibility was confirmed subsequently by fluorescence microscopy using hydrophobic fluorescent dyes and by electron microscopy using a special method developed to visualize the pulmonary surfactant PC/DPPC of Type II pneumocytes.[33,34]

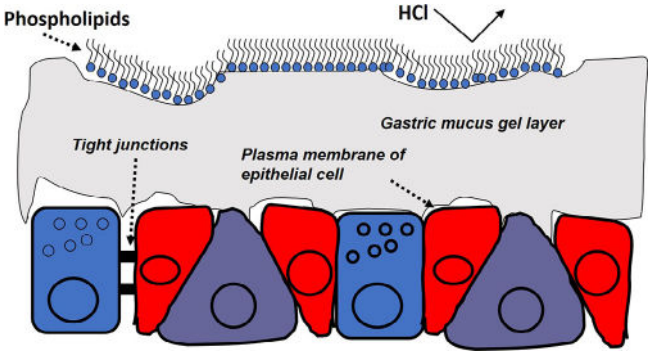

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FIGURE 3

Non-wettable surface of the gastric mucosa. Surface-active phospholipids form a monolayer hydrophobic surface at the interface of the mucus gel layer, while the luminal bulk solution serves as a non-wettable barrier to HCl. Schematic representation redrawn from Lichtenberger *et al.*[40]

In vitro and in vivo evidence that NSAIDs chemically associate with PC and that PC-NSAIDs possess reduced GI toxicity and full therapeutic activity in animal model systems

The morphological studies described above were in turn complemented by rodent and *in vitro* (Ussing Chamber and cell culture) experiments to study the effects of damaging (bile acid, NSAIDs) and cytoprotective (PGE_2) agents on gastric surface hydrophobicity and mucosal phospholipids.[35–37] This work supported the concept that the damaging agents selectively attenuated the hydrophobic properties of the surface of the gastric mucosa, whereas the cytoprotective agents restored these properties.[35–37] For example, Fig. 4 shows the acute effect of aspirin on the surface hydrophobicity of mammalian gastric mucosa. We also demonstrated, using a number of rodent ulcer model systems, that surfactant phospholipids (synthetic/DPPC or mixtures of unsaturated phospholipids and sterols) protected animals from a number of ulcer-inducing damaging agents (including NSAIDs).[38] We also determined that natural lipids (milk, soy lecithin)[39,40] that were enriched in phospholipids similarly could protect rats from NSAID-induced ulcer formation.

In order to better understand how NSAIDs acutely affect gastric surface hydrophobicity, we postulated that NSAIDs possess a strong affinity to associate chemically with PC, as both classes of molecules are amphoteric and would undergo both ionic and hydrophobic associations. This possibility was supported using biophysical measurements[38] and subsequently biochemically by NMR and computational analysis/Molecular Dynamic Simulation.[41] These studies confirmed that NSAIDs, which are weak acids carrying a negative charge at pH values above the pKa of the carboxylic acid group (in the case of aspirin, at pH > 3.5), associ-

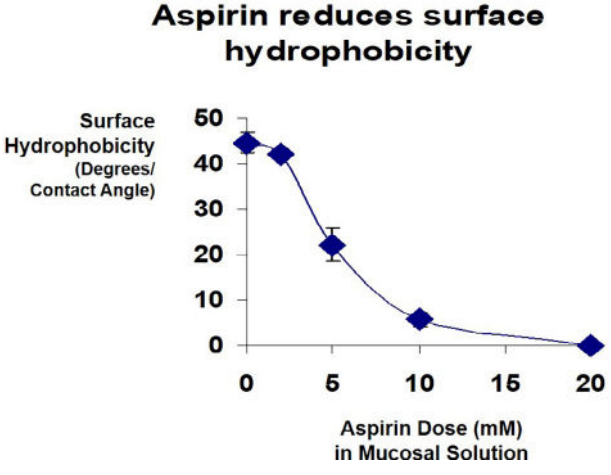

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FIGURE 4

The dose-dependent effect of luminal aspirin on the surface hydrophobicity of the mammalian gastric mucosa. Data were collected over a 30 min incubation period from tissue mounted in an Ussing Chamber. Redrawn from Goddard *et al.*[35]

FIGURE 5

Molecular association of the nonsteroidal anti-inflammatory drug (NSAID) Ibuprofen (colored purple) with 1,2-dilinoleoyl-*sn*-glycero-3-phosphatidylcholine. Nuclear magnetic resonance (NMR) showed that an ionic bond forms at the negatively charged carboxyl group of the NSAID and at the positively charged choline group of the phosphatidylcholine (PC); the fatty acid side chain of PC and the non-polar moieties of ibuprofen form a hydrophobic association. Redrawn from Lichtenberger *et al.*[41]

ate electrostatically with the positively charged choline head group of PC, while the non-polar regions of NSAIDs form hydrophobic bonds with the fatty acid side chains of the phospholipid (see Fig. 5). The possibility that NSAIDs chemically associate with phospholipids was determined by alterations in the physicochemical properties of phospholipids, as initially demonstrated in our 1995 paper in *Nature Medicine*,[38] based on changes in the organic solubility and melting point of DPPC in the presence of NSAIDs. The chemical interaction of these two classes of molecules was subsequently confirmed by our and other laboratories using quasi-elastic neutron scattering and anisotropic measurements of membrane fluidity.[42,43] NSAID-induced changes in phospholipid transition temperature were observed in these measurements, as were NSAID-induced changes in membrane thinning, permeability and cholesterol solubility, which together resulted in alterations in raft formation[44] and membrane phospholipid packing. These citated papers represent a small fraction of the >3000 papers that are recovered in a Pubmed search using the key words 'NSAID' and 'phospholipid'.

With this realization that NSAIDs chemically associate with PC and related phospholipids, we reasoned that by pre-associating NSAIDs with PC (either synthetic or purified from a PC-enriched natural product such as soy lecithin), we could effectively attenuate the GI toxicity of NSAIDs by preventing them from associating with the intrinsic phospholipids of membranes and with phospholipids on the surface of the mucus gel layer. These studies were initially confirmed in rodents (see Fig. 6)[45,46] and subsequently translated to a pilot clinical trial in which we demonstrated that purified soy PC pre-associated with aspirin reduced the NSAID-induced gastroduodenal injury or erosion of subjects as assessed endoscopically.[47]

The next important question to approach is whether PC-association altered the therapeutic activity of the NSAID. Accordingly, we evaluated a number of PC-NSAIDs (notably Aspirin-PC, Indomethacin-PC and Ibuprofen-PC) in rodent models of fever, inflammation and acute and chronic pain. These studies clearly demonstrated that the therapeutic activities of NSAIDs (antipyretic, anti-inflammatory, and analgesic) were not attenuated by PC-association and were either unchanged or even enhanced when compared to the activity of the unmodified NSAID.[40,48–50]

Evidence that PC-NSAIDs have reduced GI toxicity in human subjects

On the basis of these positive results, PLx Pharma Inc. was founded in 2003 to further develop and commercialize the PC-NSAID technology. The company decided to focus initially on developing and testing Ibuprofen-PC with a focus on its use in patients suffering from osteoarthritis. To reduce the cost of goods, we replaced purified soy PC (Phosphlipon 90G provided by Lipoid) with the company's less-costly PC-enriched soy lecithin (Phosal 35SB) containing ~35% PC. This modified Ibuprofen-PC was demonstrated to have reduced GI toxicity and full anti-inflammatory/analgesic activity in rodent models of adjuvant-induced arthritis.[40,48,49] PLx Pharma Inc. followed these animal studies with a series of Phase I and Phase II clinical trials of Ibuprofen-PC encapsulated in soft gelatin capsules, which confirmed the safety and bioequivalence of Ibuprofen-PC when compared to ibuprofen in healthy human subjects.

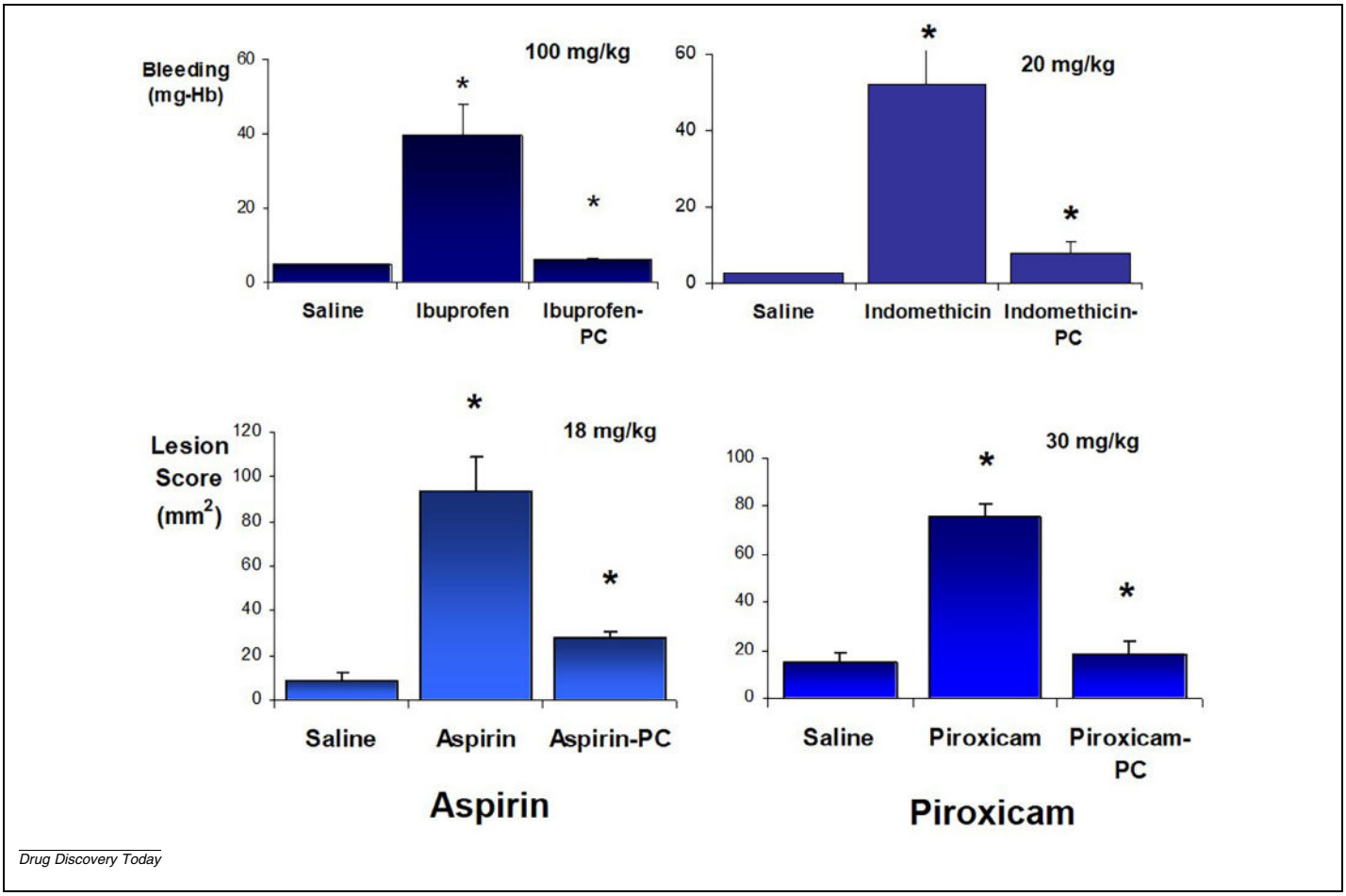

FIGURE 6

Phosphatidylcholine (PC) association reduces nonsteroidal anti-inflammatory drug (NSAID)-induced ulceration and bleeding in rodent ulcer models. Redrawn from Lichtenberger *et al.*[38]

The company filed an investigational new drug (IND) application for the novel ibuprofen, which received FDA approval. Armed with this approval, a multi-center clinical trial was set up to evaluate high-dose Ibuprofen-PC (800 mg *t.i.d*) in comparison with an equivalent dose of unmodified ibuprofen in osteoarthritic (OA) volunteers. Arthritic pain was assessed by The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scoring weekly over the 6-week trial, and endoscopic injury was assessed at baseline and at the end of the study period using the established Lanza scoring method. The results of this Phase II clinical study were published in 2008 and revealed that Ibuprofen-PC had equivalent and significant pain-reducing activity when compared to unmodified ibuprofen in OA patients and induced less endoscopic gastroduodenal injury in subjects >55 years of age.[51]

Shortly after this positive clinical trial, PLx Pharma made a corporate decision to develop another PC-NSAID, Aspirin-PC (initially called PL2200). This step was important because of the established efficacy of aspirin in reducing the incidence of cardiovascular disease[4] and the great public need for a GI-safer aspirin formulation. Millions of patients with a history of cardiovascular disease could not tolerate either 325 mg or 81 mg (baby) unmodified aspirin as recommended by cardiologists. Accordingly, the company modified the manufacture of Aspirin-PC to minimize the hydrolysis of aspirin to salicylate. As was the case with Ibuprofen-PC, we first had to establish that our novel aspirin was bioequivalent to unmodified aspirin. For aspirin, this involved obtaining confirmation that the drug maintained full anti-platelet activity, as assessed by measuring platelet aggregation and thromboxane generation. On the basis of these results, the FDA both granted approval of the submitted IND application for future clinical testing and granted Aspirin-PC full New Drug Application status in 2013. At this time, a multi-center clinical trial was designed to evaluate Aspirin-PC in comparison with unmodified aspirin in healthy volunteers over a 7-day study period. Gastroduodenal injury and platelet activity were assessed at baseline and at the end of the study by endoscopy and aggregometry, respectively. In this study we demonstrated that Aspirin-PC significantly reduced the number of gastroduodenal erosions (by 47%) and ulcers (by 71%) when compared to unmodified aspirin (see Fig. 7), and subsequently published studies demonstrated that the novel drug maintained full anti-platelet activity.[52,53].

PLx Pharma subsequently optimized the manufacturing method to suspend

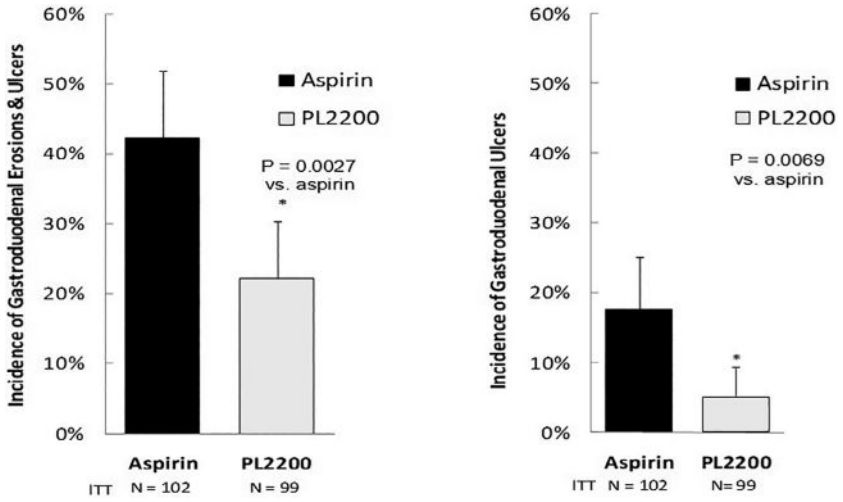

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FIGURE 7

Gastrointestinal safety of Aspirin-PC (PL2200). Summary of a clinical endoscopic study evaluating gastroduodenal ulcers and erosion in human volunteers > 50 years of age after 7 days of treatment with low-dose (81 mg) Aspirin-PC vs enteric-coated aspirin. Redrawn from Cryer *et al.*[52]

aspirin in phospholipid-enriched soy lecithin in the presence of anti-oxidants, and to limit the water-induced breakdown (hydrolysis) of the drug to salicylic acid by encapsulating the drug in hard capsules that limit atmospheric water penetration. As a consequence of this optimization of the manufacturing, the company subsequently received FDA approval for our drug at both 81 mg and 325 mg doses at an acceptable shelf-life. After some negotiation, the FDA approved the company's use of the brand name Vazalore®.

The company has subsequently performed a number of trials evaluating the anti-platelet activity of Aspirin-PC in comparison to enteric-coated Aspirin (EC-Aspirin) in obese subjects, who are prone to Type II diabetes and have a tendency to be aspirin-resistant.[53,54] The most recent trials[55,56] corroborate the superior bioavailability and anti-platelet activity (as indicated by inhibition of Thromboxane (TXB_2) generation) of Aspirin-PC (PL-2200) when compared to EC-Aspirin (see Fig. 8). The finding that obese and/or pre-diabetic subjects who are 'resistant' to the anti-platelet actions of EC-aspirin become responsive to aspirin's anti-platelet actions when switched over to Aspirin-PC strongly suggest that Vazalore® is the preferred drug to protect against cardiovascular disease in these patients (see Fig. 9). Furthermore,

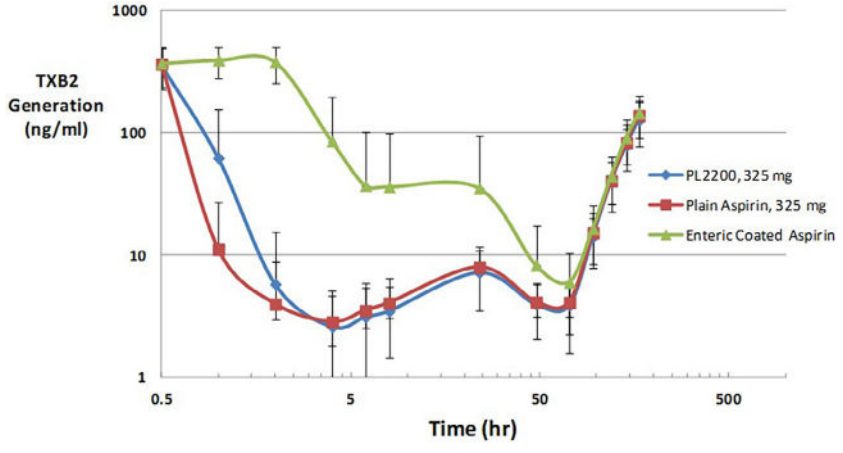

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FIGURE 8

Bioavailability of Aspirin-PC (PL-2200) when compared to enteric-coated aspirin (EC-Aspirin) and plain aspirin. Measurements of thromboxane (TXB_2), a product of COX-1 activity that induces platelet aggregation, in a clinical study of obese human volunteers show that low-dose Aspirin-PC (PL-2200) has faster onset and bioavailability than enteric-coated aspirin. Redrawn from Bhatt *et al.*[53]

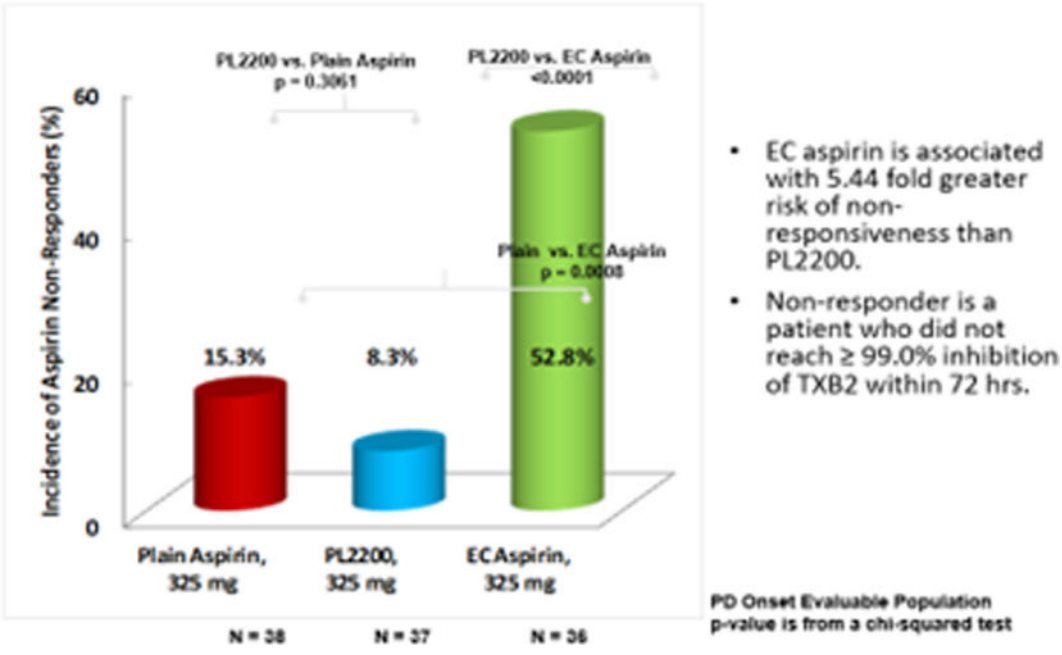

FIGURE 9

Resistance to the anti-platelet actions of Aspirin-PC (PL-2200) when compared to enteric-coated aspirin (EC-Aspirin) and plain aspirin in obese and/or pre-diabetic volunteers. Participants who were resistant to the anti-platelet actions of enteric-coated aspirin (EC-aspirin) become sensitive to the drug when switched over to Aspirin-PC (PL2200) or plain (immediate-release) aspirin.

recent papers have demonstrated that the superior anti-platelet activity of Vazalore® is not attributable to the lipid excipients of the formulation, which are inert in affecting platelet function in the absence of aspirin.[56,57] On the basis of these positive clinical trial results and optimization of manufacturing of the Aspirin-PC formulation, Vazalore® was launched in late August 2021 in 30,000 pharmacies and retail stores to be sold OTC at both 325 mg and 81 mg doses.

Of note, recent recommendations of the US Preventive Service Task Force (USPSTF) advise against the use of low-dose aspirin in individuals older than 60 years of age for primary prevention of cardiovascular disease due to the increased risk of aspirin-induced GI bleeding.[58] Therefore, a novel aspirin formulation that has an improved benefit:risk profile in older subjects could restore aspirin's role in cardiovascular disease prevention. It is for these reasons that a future large-scale clinical trial is needed to evaluate the GI safety and anti-platelet activity of Vazalore® and to determine the clinical performance of this novel formulation, especially in older patients who are at higher risk of developing aspirin-induced GI bleeds.

Conflict of interest

Dr Lichtenberger was the Scientific Founder of PLx Pharma Inc., is the primary inventor for a number of key PC-NSAID patents, and owns shares in PLx Pharma Inc.

Data availability

Data will be made available on request.

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