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REVIEW

Omega-3 polyunsaturated fatty acids as an *angelus custos* to rescue patients from NSAID-induced gastroduodenal damage

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Abstract Nonsteroidal anti-inflammatory drugs (NSAIDs) are one of the drug types frequently prescribed for their analgesic, anti-inflammatory, and antithrombotic actions, but carry a risk of major gastroduodenal damage from mild erosive changes to serious ulceration leading to fatal outcomes. From the long history of willow tree bark and its extracts being applied for the relief of pain and fever, the synthesis of acetylsalicylic acid, the development of selective cyclooxygenase 2 inhibitors (coxibs), and the identification of a G-protein-coupled receptor for prostaglandin, the popular combination regimen of an NSAID and a proton pump inhibitor was invented, but development was continued for further improvement. With regard to major NSAID adverse effects, gastrointestinal (GI) and cardiovascular (CV) risks still remained as problems to be solved. In this review, it is shown that *n*-3 polyunsaturated fatty acid (PUFA) based NSAIDs can be an *angelus custos*, supported with facts that an intake of essential *n*-3 PUFAs orchestrates concerted protective actions against two notorious side effects of NSAIDs, the aforementioned GI risk and CV risk of NSAIDs. Since pills containing *n*-3 PUFAs, omega-3-acid ethyl ester capsules (Lovaza, Omarcor), have already been safely prescribed to prevent atherosclerosis through lessening lipid burdening, the introduction of a drug delivery system such as a gastroretentive form of *n*-3 PUFA based NSAIDs will highlight newer hope for GI safety under the guarantee of reduced CV risk. Because *n*-3 PUFAs have been proven to attenuate cytotoxicity, inhibit lipid-raft-associated harmful signaling, and relieve oxidative stress relevant to NSAIDs, *n*-3 PUFA based NSAIDs will be next-generation GI-safe NSAIDs.

Keywords Nonsteroidal anti-inflammatory drug · Gastrointestinal risk · *n*-3 polyunsaturated fatty acid · Lipid raft · *n*-3 polyunsaturated fatty acid based nonsteroidal anti-inflammatory drug

Abbreviations

COX	Cyclooxygenase
Coxib	Cyclooxygenase 2 inhibitor
CV	Cardiovascular
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
ER	Endoplasmic reticulum
GI	Gastrointestinal
LTB_4	Leukotriene B_4
NSAID	Nonsteroidal anti-inflammatory drug
PGE_2	Prostaglandin E_2
PGI_2	Prostacyclin
PPI	Proton pump inhibitor
PUFA	Polyunsaturated fatty acid
ROS	Reactive oxygen species
RvD1	Resolvin D1

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Unmet medical need regarding adverse effects associated with nonsteroidal anti-inflammatory drugs

Although recent studies have shown a profound decrease in the number of hospitalizations due to upper gastrointestinal (GI) tract complications owing to widespread use of proton pump inhibitors (PPIs), safer nonsteroidal anti-inflammatory drug (NSAID) prescription including cyclooxygenase (COX)-2 inhibitors (coxibs), combination with some gastroprotective medications, and decreased prevalence of *Helicobacter pylori* infection, improvement is still mandatory to achieve higher GI safety, lower cardiovascular (CV) risk, and rescue of patients from renal complications [1]. With inhibition of COX-1 with aspirin or ibuprofen, NSAIDs result in degradation of the protective mucous layer of the stomach and induce various degrees of damage ranging from relatively mild nausea and epigastric pain to severe perforation and bleeding ulcer [2], as well as various degrees of CV and renal toxicity. Naproxen is the best option in patients with high CV risk, but imparts moderate GI risk. On the other hand, patients taking aspirin are a great challenge for treatment, since interaction with frequently prescribed NSAIDs such as ibuprofen and naproxen may alter the antiplatelet effect of aspirin, representing a potential clinical problem of increased risk of GI bleeding. Changing NSAID treatment to diclofenac or celecoxib may also not be an option in patients with a history CV events [3]. Coxibs reduce endothelial production of prostacyclin (PGI_2), while leaving the platelet production of thromboxane A2 intact, by which increasing degrees of selectivity for COX-2 are associated with augmented CV risk and inversely decreasing degrees of selectivity for COX-2 (COX-1 inhibitor) are associated with augmented GI risk. Although risk evaluation should be considered before the use of safer and efficacious NSAIDs in the clinic at the individual patient level, in practice, since different clinical scenarios involving these three factors associated with the presence of different GI and CV risk factors exist, the best solution is tailoring each individual GI and CV risk [4]. In the following sections, we give a brief description of adverse effects of nonselective NSAIDs.

GI risk

Although whether NSAID intake in the presence of *H. pylori* infection may further increase the risk of peptic ulcer caused by the presence of only one risk factor is still a matter of debate, *H. pylori* infection, NSAID use, or low-dose aspirin use increased the risk of development of peptic ulcer as well as GI complications significantly and independently [5]. With regard to gastric damage, NSAID-induced enteropathy has gained much attention in the last few years with the introduction of capsule endoscopy and enteroscopy. NSAIDs increase the risk of lower GI tract bleeding and perforation to a similar extent as that seen in the upper GI tract, and coxibs have the same beneficial effects as nonselective NSAIDs but with less GI toxicity in the upper GI tract and probably in the lower GI tract, whereas PPI combination aggravated intestinal damage due to changes in gut microbiota [6, 7]. In spite of these limitations, the strategy of choice for GI risk reduction with NSAIDs is the combination of conventional NSAIDs with PPIs or coxibs because they were reported to reduce the risk [8]. In the pursuit of greater safety under guaranteed benefits of NSAIDs, the development of NO-releasing, H_2S-releasing, and phosphatidylcholine-associated NSAIDs is continuing, but is still in the preclinical stage.

CV risk

The discovery of two COX isoforms—COX-1 constitutively expressed in normal tissues, and COX-2 induced at sites of inflammation—led to the development of coxibs with the hope of significantly reducing the GI toxicity mentioned before. However, increased knowledge of the physiological roles of COX-2 in a variety of tissues, especially after COX-2 knockout mice showed renal and CV abnormalities, and the withdrawal of rofecoxib and valdecoxib from the market because of reported CV toxicity have challenged the benefits of coxibs [9]. NSAID usage generally imposed the risk of CV events including myocardial infarction or cerebrovascular accident whereas low-dose aspirin is usually used to decrease the risk of CV disease for high-risk patients [10], whereas NSAID use is associated with excess risk of death and myocardial infarction by use of coxibs. The vessel-constricting compound thromboxane A2 stimulates activation of platelets and increases platelet aggregation, and blood vessels produce the anticlotting compound PGI_2. To balance the opposing actions of clotting and blood flow, COXs regulate thromboxane A2 and PGl_2 levels during blood vessel injury. Since coxibs reduce the amounts of PGI_2, they increase the risk of myocardial infarction. In addition, increases in vasoconstriction caused by coxibs can also lead to high blood pressure and renal insufficiency [11].

Renal risk and other risks

Long-term use of NSAIDs can lead to impaired renal blood flow and chronic renal damage by continuous inhibition of prostaglandins [12]. Since prostaglandins are important for proper blood vessel function within the kidneys [13], high-dose NSAID therapy is associated with chronic kidney disease. NSAID-associated chronic kidney disease leads to high blood pressure, salt and water retention, and

electrolyte imbalances [13]. The major cause of NSAID-associated renal damage is the contribution of NSAIDs to mitochondrial dysfunction, resulting in excessive production of highly reactive free radicals and subsequent tissue damage [14]. Also, NSAIDs caused liver toxicity relevant to oxidative stress in vascular tissue [15]. Hematological complications of traditional NSAIDs are relatively rare, but NSAIDs increase the bleeding time of blood by inhibition of thromboxane A2. Consequently, the decreased platelet aggregation and the prolonged bleeding time further aggravate GI complications. Phenylbutazone use and indomethacin use are sometimes associated with agranulocytosis [16].

Safer NSAIDs to cover the aforementioned adverse effects

In addition to synthetic prostaglandins, two gaseous mediators—nitric oxide (NO) and hydrogen sulfide (H_2S)—exert protective effects in gastric mucosa. The development of NO- or H_2S-releasing NSAIDs is continuing [17, 18]. NO-releasing (or NO-donating) NSAIDs have been used to inhibit NSAID-induced leukocyte adherence by release of NO, and H_2S-releasing NSAIDs have been used to inhibit NSAID-induced vasoconstriction and ischemia by release of H_2S. All of these NSAIDs rescue patients from GI and CV risk as well as renal complications. In addition, efforts to obtain drugs able to inhibit both 5-lipoxygenase and COX, the so-called dual-acting anti-inflammatory drugs [19], have continued because dual-acting anti-inflammatory drugs retain the activity of classic NSAIDs, while avoiding their main drawbacks [20]. Currently, various structural families of dual-acting anti-inflammatory drugs have been designed, and several compounds are under clinical investigation [21], although humans have taken almost 2,000 years to develop coxibs, starting from willow tree bark to the development of aspirin [22] (Figs. 1, 3).

fat-1 transgenic mice producing *n*-3 polyunsaturated fatty acids exhibited protection from NSAID damage

In 2004, Kang et al. [23] first generated *fat-1* transgenic mice. These mice engineered to carry the *fat-1* gene, which encodes an *n*-3 desaturase, from the worm *Caenorhabditis elegans* can add a double bond into an unsaturated fatty acid hydrocarbon chain and can convert *n*-6 polyunsaturated fatty acids (PUFAs) to *n*-3 PUFAs (Fig. 2). Mammals cannot naturally produce *n*-3 PUFAs, and so must rely on a dietary supply—from fish, walnuts, olive oil, etc. Since *fat-1* transgenic mice carry an abundance of *n*-3 PUFAs but low levels of *n*-6 PUFAs in their organs and tissues in the absence of dietary *n*-3 PUFAs, this resulted in significant benefits with regard to clinical diseases and these mice emerged as a new model for omega-3 research [24], as exemplified as follows: reduced carcinogenesis and cancer prevention [25–27] and protection from metabolic diseases [28, 29], osteoarthritis [30], several ischemic injuries [31, 32], nonalcoholic steatohepatitis [33, 34], and oxidative injury [34]. Since Lim et al. [35] showed inhibition of hepatocellular carcinoma growth through blocking of β-catenin and COX-2 and these anticancer effects shared the molecular mechanisms of NSAIDs, our group hypothesized that *n*-3 PUFAs might impose NSAID-sparing effects. *fat-1* mice (Fig. 2) showed significant rescuing outcomes such that indomethacin-induced gastric ulcers as well as intestinal ulcers were significantly ameliorated. These rescuing actions of *n*-3 PUFAs were proven to be mediated through glucose-regulated protein 120, after which blocking significantly apoptotic signals of indomethacin, scavenging oxidative stress of indomethacin, and attenuating inflammatory mediators as well as relieving ischemic conditions. As for NSAID-induced gastric ulcerations, recently much attention has been paid to intestinal injuries. Although a PPI can prevent NSAID-induced gastric ulcerations, it rather aggravated intestinal ulcerations through dysbiosis. Therefore, the emergence of *n*-3 PUFA based NSAIDs can be an escape from NSAID-induced GI damage (Fig. 3).

Molecular mechanisms for how *n*-3 PUFAs rescue patients from NSAID-induced GI damage

Attenuating NSAID-associated apoptosis

The main pharmacological action of NSAIDs is through the inhibition of prostaglandin synthesis via the suppression of COX activity, but indiscernible decreases of the levels of gastroprotective prostaglandin E_2 (PGE_2) have been regarded as a factor contributing to GI toxicity. There have been many publications explaining COX-independent mechanisms relating to GI toxicity, one of which is that NSAIDs themselves can trigger apoptosis mediated by endoplasmic reticulum (ER) stress [36]. Since ER stress prevails after administration of NSAIDs and autophagy is known as a phenomenon following ER stress, autophagic cell death can be a factor responsible for NSAID-induced GI toxicities. Autophagic cell death is inducible in response to cellular stresses, including nutrient deprivation and oxidative stress [37]. Tsutsumi et al. [36] first examined the association between ER stress response and NSAID-induced apoptosis. Exposure of gastric mucosal cells to indomethacin induced glucose-regulated protein 78 and C/EBP homologous transcription factor as well as activating transcription factor 6, activating transcription

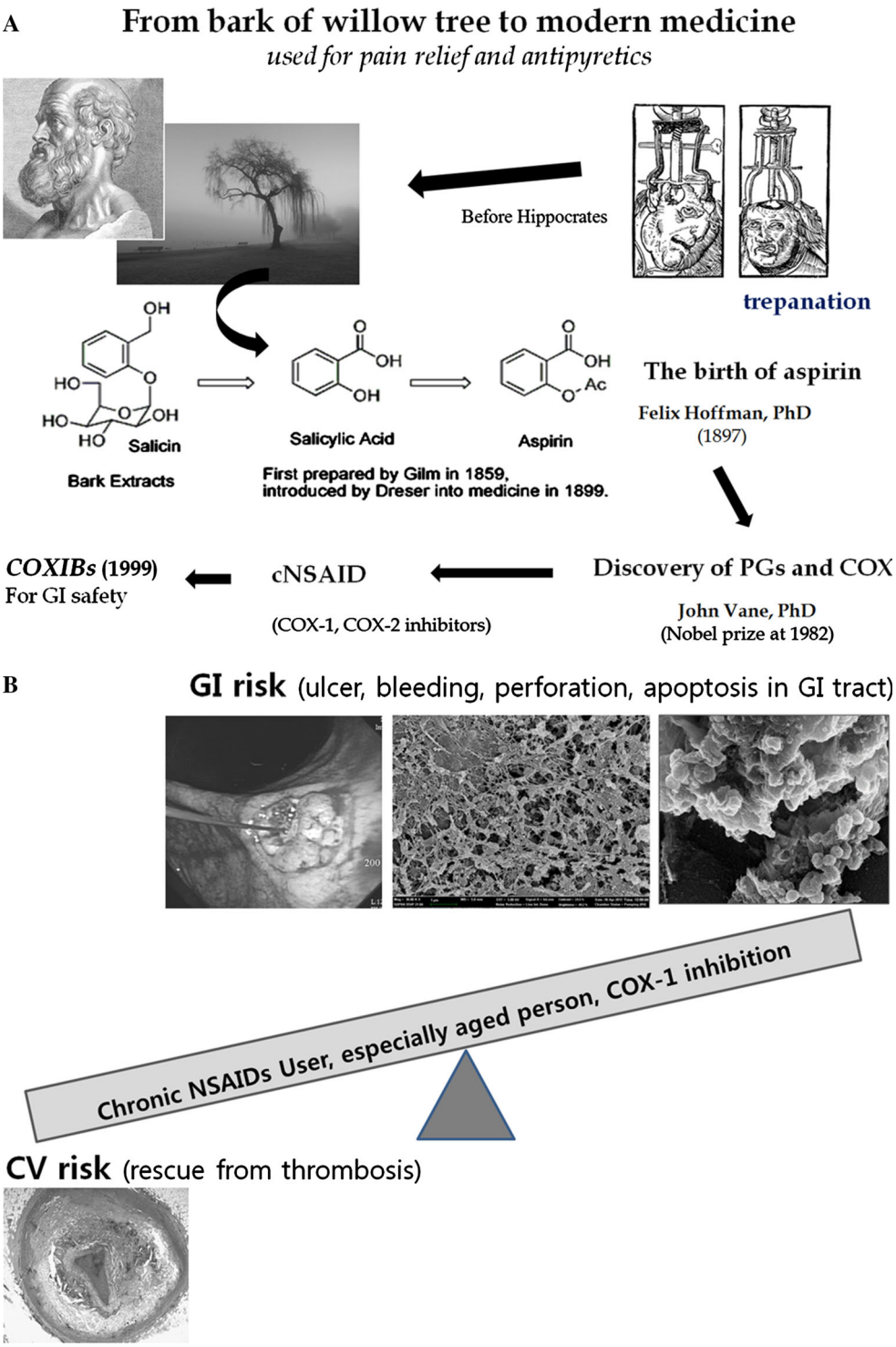

Fig. 1 Development of gastrointestinal (*GI*)-safe nonsteroidal anti-inflammatory drugs (NSAIDs): progression from willow tree bark to modern medicine. **a** History of development of cyclooxygenase 2 (*COX-2*) inhibitors (*COXIBs*) starting from ancient trepanation to willow tree bark extracts and synthesis of acetylated salicylic acids. **b** Double-edged sword of NSAIDs, i.e., higher GI risk, but lower cardiovascular (*CV*) risk with administration of conventional nonselective NSAIDs, whereas COXIBs lower GI risk, but increase CV risk. Celecoxib, showing 4–5 of COX-2 selectivity, exhibits greater protection from either cerebral infarction risk or CV risk. *cNSAID*, *COX* cyclooxygenase, *COX-1* cyclooxygenase 1, *PG* prostaglandin

factor 4, and X box binding protein 1, suggesting that ER stress response seems to be generally involved in NSAID cytotoxicity irrespective of the kind of NSAID. Chen et al. [38] additionally found that the cytotoxic outcome after use of different pharmacological agents of various COX-2 inhibitory potencies was closely aligned with these agents' ability to trigger ER stress. Therefore, ER stress seems to be one of the fundamental COX-independent mechanisms of NSAID-induced gastric mucosal cytotoxicity. Another mechanism by which NSAIDs induce apoptosis is by reducing the levels of survivin, a known apoptosis inhibitor [39]. Overexpression of survivin effectively inhibited

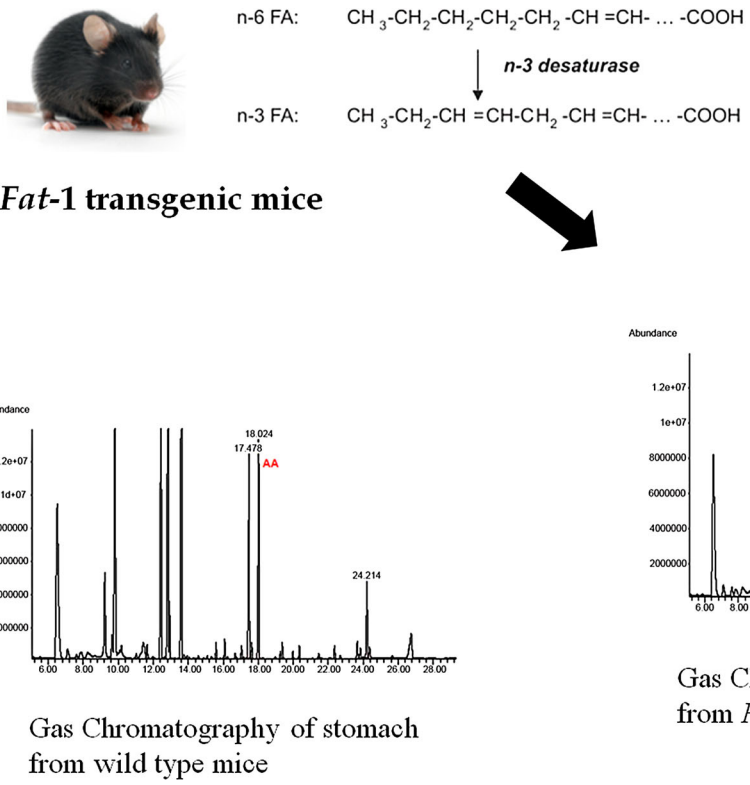

Fig. 2 *n*-3 polyunsaturated fatty acids (PUFAs); essential fatty acids (*FA*) exerting various beneficiary actions for unmet medical needs, by which *fat-1* transgenic (*TG*) mice produced *n*-3 PUFAs through overexpressed *n*-3 desaturase. *fat-1* transgenic mice showed significant protection from indomethacin-induced gastroduodenal damage, protection from which was significantly correlated with gastric contents of *n*-3 PUFAs [α-linolenic acid (*ALA*), docosahexaenoic acid (*DHA*), and eicosapentaenoic acid (*EPA*)]. On gas chromatography of stomach homogenates, *n*-3 PUFAs were generated owing to the presence of the *n*-3 desaturase transgene in *fat-1* transgenic mice, whereas they were not detected in wild-type littermates (our unpublished data). *AA* arachidonic acid

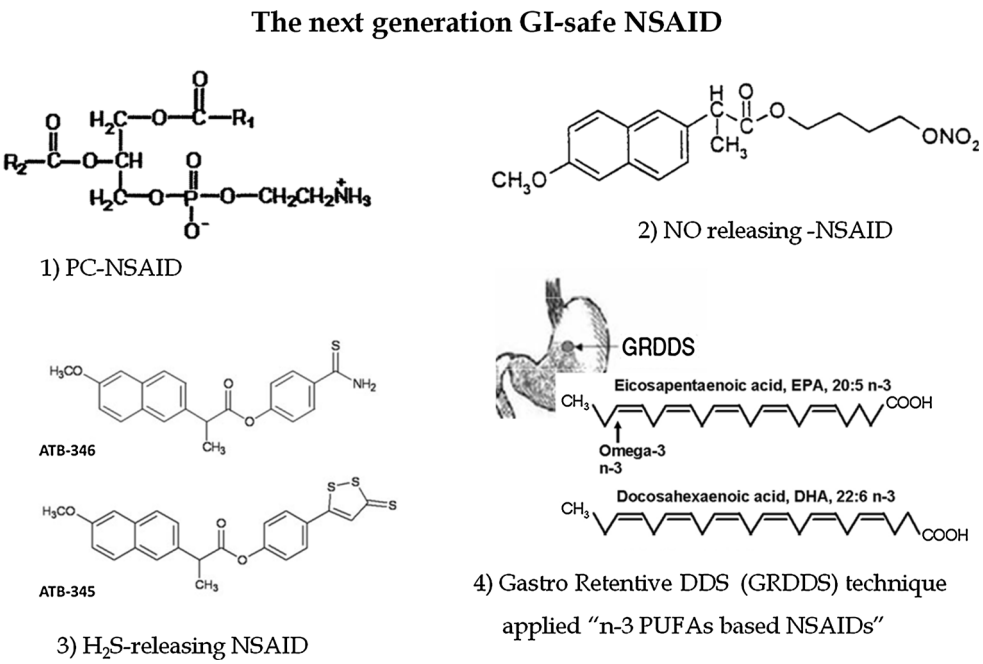

Fig. 3 *n*-3 polyunsaturated fatty acid (*PUFA*) based nonsteroidal anti-inflammatory drugs (*NSAIDs*) as next-generation gastrointestinal (*GI*)-safe NSAIDs. Phosphatidylcholine (*PC*)-associated NSAIDs, NO-releasing NSAIDs, and H_2S-releasing NSAIDs are all in clinical trials, and we strongly believe that *n*-3 PUFA based NSAIDs used with a gastroretentive drug delivery system (*GRDDS*) might soon be next-generation GI-safe NSAIDs

NSAID-induced apoptosis in cultured RGM-1 cells [40]. However, small interfering RNA depletion of LC3 blocked downregulation of survivin by NSIADs, indicating that survivin functions downstream of vacuole formation to regulate the sulindac sulfide induced autophagic cell death pathway [40]. NSAIDs can also mediate apoptosis by inducing the activation of caspases, a family of proapoptotic cysteine proteinases which typically exist as latent zymogens in cells. Activation of these proteins by proapoptotic signals initiates a caspase cascade whereby initiator caspases specifically activate other executioner-type caspases [41].

Enhancing mucosal resolution

It is well known that *n*-3 PUFAs have anti-inflammatory and antiapoptotic affects. Resolvins are a novel family of lipid mediators derived from eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), both of which are *n*-3 PUFAs with anti-inflammatory and antiapoptotic effects and are classified with two distinct structural forms, the E series and the D series, respectively. In the presence of aspirin, EPA is converted by acetylated COX-2 in vascular endothelial cells to (18*R*)-hydroperoxyeicosapentaenoic acid, which is the precursor of resolvin E1, catalyzed by 5-lipoxygenase [42] or cytochrome P450 monooxygenase [43]. DHA is converted by 15-lipoxygenase type I to 17-hydroxydocosahexaenoic acid, which is the precursor of the D series resolvins such as resolvin D1 (RvD1) and resolvin D2 during acute inflammation. Aspirin-acetylated COX-2 is the precursor of (17*R*)-hydroxydocosahexaenoic acid, which is oxygenated by 5-lipoxygenase, which results in the generation of (17*R*)-RvD1 [44–46]. The protective effect of *n*-3 PUFAs against NSAID-induced ER stress and autophagy has not been elucidated yet. However, a recent study reported that RvD1 prevented ER-stress-induced hepatic apoptosis and lipid accumulation through the JNK-dependent pathway without inducing significant changes in the level of ER stress [47]. In addition, EPA attenuated statin-induced ER stress, JNK activation, and toxicity in cultured myoblast cells, and peroxisome-proliferator-activated receptor δ may be mechanically involved in the effects of EPA [48]. Also, the administration of DHA has therapeutic potential in reducing ER stress, abnormal protein accumulation, and neurological deficits in chronic neuronal damage [49]. These results provide reliable evidence that *n*-3 PUFAs have cytoprotective effects through attenuation of NSAID-induced ER stress and autophagic cell death.

Attenuating NSAID-induced inflammatory actions

Traditional NSAIDs such as aspirin and indomethacin are widely used for the relief of pain and inflammation. However, their use is limited by their gastric toxicity. NSAID-induced gastric damage is due in large part to COX inhibition [50]. Whereas COX-1 is involved in the biosynthesis of prostaglandins that regulate mucosal blood flow and epithelial mucus and bicarbonate secretion, prostaglandins derived from COX-2 are involved in the reduction of leukocyte adherence and re-epithelialization of gastric cells [51]. Even with the introduction of coxibs, gastric injury has not been abolished [52]. In light of these findings, there is a high clinical need for therapies that prevent the gastric toxicity of NSAIDs. Gastroprotective effects of fish oil have been reported in gastric ulcers induced by ethanol [53, 54], aspirin [55, 56], indomethacin [57], dexamethasone [58], cold-restraint stress [59], and pyloric ligation [56]. Several mechanisms have been suggested to be involved in the gastroprotective effects of fish oil, including a decrease in gastric acid secretion and lipid peroxidation as well as an increase in the levels of antioxidant enzymes during pyloric ligation and cold-restraint stress in rats [56]. Although several studies have shown the gastroprotective effect of *n*-3 PUFAs and have reported some potential mechanisms behind this effect, it is less understood which pure compounds of fish oil are responsible for this effect. DHA is an *n*-3 PUFAs present in fish oil. DHA exhibits several actions, such as anti-inflammatory, neuroprotectitve, and cardioprotective effects [60, 61]. DHA also inhibits dextran sulfate sodium induced colitis in mice. Furthermore, DHA downregulates expression of tumor necrosis factor α and interleukin-1β increased by colitis [62]. Together, these findings suggest that DHA may be a pure compound of fish oil responsible for the gastroprotective actions. NSAID-induced gastric damage is due in large part to COX inhibition, which results in inhibition of synthesis of prostaglandins. Prostaglandins participate in the signal of gastric protective factors; PGE_2 is involved in the increase of mucus secretion and gastric blood flow. NSAID-induced gastric mucosal injury includes inhibition of mucosal prostaglandin synthesis. It has been demonstrated that PGE_2 increases mucus and bicarbonate secretion and gastric blood flow and decreases acid secretion. Thus, an inhibition of the synthesis of prostaglandins, including PGE_2, by NSAIDs would block the gastroprotective actions of PGE_2. Furthermore, leukotriene B_4 (LTB_4) has been implicated in NSAID-induced leukocyte adhesion [63], and inhibition of synthesis of LTB_4 resulted in a significant reduction of NSAID-induced gastric injury [64, 65]. Acute DHA administration blocks the indomethacin-induced increase of LTB_4 levels [65]. *n*-3 PUFAs such as DHA may exert beneficial effects by competing with *n*-6 PUFAs such as arachidonic acid for the production of lipid inflammatory mediators such as leukotriene [66]. Furthermore, it has been shown that human ingestion of fish oil leads to a

decrease in LTB_4 levels and an increase in the levels of leukotriene B_5, a weak inducer of inflammation and a weak chemotactic agent [67].

Enhancing host defense phase 2 enzyme response

NSAIDs can induce damage or apoptosis by upregulating the generation of reactive oxygen species (ROS) and by inducing oxidative stress coupled with many proapoptotic signals such as nuclear factor κB inhibition and mitogen-activated protein kinase activation [41]. However, the cellular prooxidant behavior of NSAIDs has often been the subject of controversy owing to conflicting reports that NSAIDs such as indomethacin and sulindac scavenge ROS and exert a cytoprotective and antioxidant effect in cells [68, 69]. It has also been shown that low doses of aspirin can confer long-term cytoprotective resistance against H_2O_2-induced oxidative stress in *Saccharomyces cerevisiae* cells [70]. Nevertheless, it has been accepted that NSAIDs can induce the proapoptotic accumulation of ROS in mammalian cells. The molecular mechanisms underlying NSAID-induced proapoptotic generation of ROS have not yet been fully elucidated. However, it is well known that mitochondria, a major source of cellular ROS [71] and a central component of the apoptotic machinery, are profoundly affected by NSAIDs [72]. For example, the aspirin metabolite salicylate has been shown to inhibit the mitochondrial electron transport chain in mammalian cells by interacting with an Fe–S cluster of complex I through its *o*-hydroxyl group, inducing ROS accumulation and oxidative stress, and finally causing proapoptotic events such as mitochondrial permeability transition and cytochrome *c* release [73]. Likewise, aspirin-induced cell cycle arrest and apoptosis of HepG2 hepatoma cells were shown to be induced by ROS accumulation and increased oxidative stress, and were accompanied by severe mitochondrial dysfunction such as the inactivation of electron transport chain proteins and aconitase [74].

Antioxidative actions

The long-chain *n*-3 PUFAs in fish oil, especially EPA and DHA, have well-known antioxidant properties [75]. Recent studies showed that 3 weeks of a diet enriched in *n*-3 PUFAs was sufficient to cause increased antioxidant enzyme expression and decreased mitochondrial ROS generation in mouse heart [76] and human heart [77]. These findings implicate peroxisome-proliferator-activated receptor γ activation and augmented mitochondrial fatty acid oxidation as possible therapeutic mechanisms of *n*-3 PUFAs, and suggest that *n*-3 PUFAs exert beneficial effects by enhancing antioxidant capacity. Providing more than 3 g of marine *n*-3 PUFAs per day to healthy volunteers resulted in decreased production of ROS by blood neutrophils stimulated with different agents [78] and high-dose marine *n*-3 PUFAs decreased hydrogen peroxide production by human monocytes [79]. Rees et al. [80] identified an EPA-dose-dependent decrease in the number of blood neutrophils producing superoxide in elderly subjects, and therefore fish oil supplements decreased ROS production by neutrophils. In addition, DHA and EPA showed antioxidative properties through activation of nuclear-factor-like 2, accompanied by induction of antioxidant enzymes such as heme oxygenase 1, which may explain the mechanism of the cytoprotective effects of DHA and EPA [81]. There have been a number of clinical trials of fish oil in patients with inflammation. Most of these trials report some clinical improvements—for example, improved patient-assessed pain, decreased morning stiffness, fewer painful or tender joints, and decreased use of NSAIDs—and when the trials have been pooled in meta-analyses, statistically significant clinical benefit has emerged.

Cell fluidity and lipid raft changes

The molecular basis of both the therapeutic benefit of and the pathogenesis caused by NSAIDs has been attributed to the ability of these drugs to inhibit COX, the rate-limiting enzyme in the biosynthesis of a family of prostaglandins, which plays a central role in the mediation of inflammatory, nociceptive, and cytoprotective cellular pathways [82]. One of the alternative mechanisms by which NSAIDs can affect both biological and cytotoxic responses is by interacting with cellular membranes and altering their biophysical properties [83]. NSAIDs are highly amphiphilic and strongly associate with lipid membranes, which influence localization, structure, and function of membrane-associating proteins and actively regulate cell signaling events [84, 85]. Thus, it is possible that NSAIDs regulate diverse cell functions by altering microenvironments within the membrane through changes in the fluidity, permeability, and biomechanical properties of cell membranes. The interaction of NSAIDs with phospholipid and the ability of the drugs to partition into the membranes can induce marked changes in the permeability, fluidity, and biomechanical properties of these membranes [83–85], after which these NSAID–phospholipid-induced changes will not only increase the permeability of biological membranes to potentially damaging ions, H^+ and macromolecules, e.g., enterotoxins, but will also induce an instability of the membranes owing to the formation of unstable pores as indicated by a decrease in lysis [86]. Thus, this chemical association between NSAIDs and zwitterionic phospholipids provides us with a molecular understanding of the pH-dependent effects of this potent class of anti-inflammatory drugs to induce injury to the GI

mucosa, resulting in ulcer formation and bleeding, which may lead to an increased risk of developing potentially life-threatening hemorrhage. Nunes et al. [87] demonstrated that in general NSAIDs interact with polar head groups of the membrane phospholipids when the drugs are charged and penetrate deeper into the membrane when the drugs are neutral and may result in the development of pores in the bilayer. Further, they demonstrated that these interactions appear to be dependent on the lipid phase [88]. One central molecular target of *n*-3 PUFAs involves alterations to the plasma membrane. DHA has been shown to have significant effects on plasma membrane properties, including altering membrane fluidity, phase behavior, permeability, fusion, flip-flop, and resident protein activity [89, 90]. Furthermore, recent evidence suggests that DHA can perturb specialized regions of the plasma membrane known as lipid rafts [91–93]. Lipid rafts are small, 10–200-nm, heterogeneous microdomains that are enriched in cholesterol, sphingolipids, and saturated acyl chains [94]. Lipid rafts serve as signaling platforms by compartmentalizing plasma membrane proteins and lipids. In response to stimuli, nanometer-scale domains can coalesce and display high molecular order [95]. Signaling pathways emanating from lipid rafts mediate a variety of mitogenic, metastatic, and other tumor-promoting cellular activities, and these pathways are often hyperactivated in cancer [96]. Additionally, chronic inflammation, central to the process of tumorigenesis [97], involves excessive T-cell activation, which is regulated by lipid rafts. The property of *n*-3 PUFAs in the regulation of lipid rafts, and lipid-raft-mediated signaling may protect cells against NSAID-induced changes in membrane composition resulting in altered cell function. *n*-3 PUFAs enhanced the clustering of lipid rafts to form large raft domains [92] and induced modifications of lipid raft composition [98, 99], which are significant because cholesterol and sphingomyelin are major building blocks of lipid rafts that promote the formation of hydrophobic-liquid-ordered molecular packing. In addition, lipid raft organization modified by *n*-3 PUFAs, i.e., altered the physical properties of biological membranes, leads to altered cell signaling and function [100, 101]. These studies suggest a specificity of *n*-3 PUFAs in disruption of lipid rafts by NSAIDs. However, further work is needed to clarify the effects of EPA and DHA on lipid raft perturbation.

Perspective of "*n*-3 PUFA based NSAID" development to cover GI and CV risk of NSAIDs

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. One approach to attenuating the damaging action of NSAIDs on the surface of the GI mucosa, which is caused by the drugs interacting with phospholipids present either in the bilayer or in the presumptive monolayer coating the mucus gel lining, is to preassociate the NSAID with either synthetic or natural phosphatidylcholine. Lichtenberger et al. [86, 102] developed a noncovalent complex between natural phosphatidylcholine and an NSAID. The association of the NSAID and phosphatidylcholine 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 [83]. This approach reduces the affinity of a luminal NSAID to interact with intrinsic phospholipids of the GI mucosa, fortifying the tissue's barrier properties, while promoting the transit of the drug across the mucosa into the blood owing the increased lipophilicity of the conjugate. This appears to be an effective strategy to reduce the GI toxicity of a number of NSAIDs, notably aspirin, ibuprofen, naproxen, and indomethacin, while maintaining their bioavailability and therapeutic efficacy in both rodent models and pilot clinical endoscopic trials [102, 103]. In this review, we have presented the beneficial effects of *n*-3 PUFAs on NSAID-induced apoptosis, inflammation, and oxidative stress. Therefore, the development of *n*-3 PUFA based NSAIDs or a coadministration regimen may guarantee clinical safety of NSAID administration, the efficacy of which might soon be potentiated by the introduction of a gastroretentive drug delivery system (Fig. 3).

Conclusion

To rescue patients from NSAID-induced GI adverse effects, several approaches had been adopted, including coxibs, co-medication of PPI or synthetic prostaglandin analogs, and efficacious phytoceuticals [104]. Recently, newer NSAIDs to alleviate GI and CV pitfalls inherent to current therapy options across the entire NSAID class are mandatory and are under active development. In detail, NO- or H_2S-releasing NSAIDs, phosphatidylcholine-combined formula, injectable formulation, potent natural phytoceuticals, two orally administered NSAID formulations (e.g., naproxen plus PPI or ibuprofen plus oxycodone), three topical NSAID formulations (e.g., diclofenac patch, gel, and solution), novel intra-articular extended-release

NSAIDs, co-prescription of gastroprotective agents, and nanoformulations of submicron NSAIDs featuring delivery of decreased doses will be the future of NSAID therapy [105–107]. Our investigation group strongly believes our novel formula, *n*-3 PUFA based NSAIDs, can achieve (1) an NSAID-sparing effect based on the authentic anti-inflammatory action of *n*-3 PUFAs, (2) guaranteed safety of GI, CV, and renal systems, (3) correction of all derangements relevant to NSAIDs, (4) significant protection of the whole GI tract, and (5) concerted mode of action against arthritis. However, more purified and concentrated *n*-3 PUFAs should be generated to successfully support the authentic action of NSAIDs. Since the in-hospital case fatality for upper and lower GI tract complication events has remained constant despite the new therapeutic and prevention strategies [6], more effort to develop more efficacious but safer NSAIDs should be pursued in the clinic in the era of the super aging society.

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Conflict of interest The authors declare that they have no conflict of interest.

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