Introduction

The nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of pain and inflammation in rheumatic disorders and osteoarthritis [1]. These drugs are also used as antineoplastic agents and to prevent and treat ischemic heart disease [2]. Unfortunately, NSAIDs also contribute to severe gastrointestinal complications [3-7]. The NSAIDs-related gastrointestinal damage is very common. It is estimated that, up to 20% of the patients using NSAIDs on a regular basis develop gastropathy, while, up to 33% of the patients taking it for an extensive period generate gastric or duodenal ulcers [1, 7]. The NSAIDs-related gastropathology is considered as a “silent epidemic” and, therefore, has been an area of extensive research [7, 8]. Several mechanisms have been proposed to address this complicated disorder. Both prostaglandin-dependent, as well as prostaglandin-independent mechanisms, are involved in the pathogenesis of NSAIDs-induced ulceration. In the prostaglandin-independent part NSAIDs-induced production of reactive oxygen species (ROS), and inflammation are considered as major players in the pathogenesis of gastric mucosal injury [5, 9]. Essentially these are not very different and independent phenomenon. In fact, these are very intricately linked and regulate themselves through positive feedback loops. Besides directly damaging gastric tissue, oxidative stress induces inflammation whereas inflammation, when sets off, induces oxidative stress in various ways. Even complex cross talks exist in between the prostaglandin dependent and independent pathways. Hence managing the NSAIDs related gastropathy is somehow a tricky problem. In this scenario, antioxidants and/or anti-inflammatory compounds seem to be effective against the NSAIDs-induced gastropathy. Here we are going to focus briefly on the recent trends in the field of gastropathy and the mechanistic basis of action of the contemporary gastroprotective agents.

NSAIDs induced gastropathy: oxidative stress and...
**antioxidants**

The role of oxidative stress and inflammation in the pathogenesis of indomethacin-induced gastric damage is well established [6, 7, 10]. A well-known NSAID, indomethacin (IND) was found to bind to a site adjacent to the complex I and ubiquinone of mitochondrial electron transport chain [111]. This binding generates ROS and ROS in turn inactivate mitochondrial aconitase [10]. Inactivation of mitochondrial aconitase results in the production of free iron, which in turn generates more mitochondrial •OH [1, 6, 12-14]. Mitochondrial dysfunction, formation of the mitochondrial permeability transition pore, and generation of mitochondrial oxidative stress (MOS) is associated the oxidative stress [1, 10]. However, Bindu et al. showed that the gastric mucosa tries to combat the stress by inducing HO-1 and translocating it to mitochondria for the period of gastric mucosal injury to favor repair mechanisms [15]. After a detail mechanistic study, they confirmed that the mitochondrial translocation of HO-1 detoxifies accumulated heme, which is a normal phenomenon during the gastric damage [15]. They also proposed a positive feedback loop where heme may activate nuclear translocation of NF-E2-related factor 2 to induce HO-1 through ROS generation [15].

Extensive research showed that, not only for protecting against gastric mucosal injury, antioxidants might be effective in inhibiting the progression of gastric ulcer that may lead to protection against oxidative damage to lipids, proteins, and the thiol-dependent antioxidant defense systems. In the recently published paper in the Biochimica et Biophysica Acta (BBA) - General Subjects, we reported that in the gastric tissue, IND significantly augmented ROS production and simultaneously reduced the activity and/or level of the antioxidant enzymes SOD2, GST and catalase [10]. Whereas, pretreatment with the morin reduced the ROS production and restored the activity and/or level of the antioxidant enzymes [10]. Here it is worth mentioning that IND indirectly modulate catalase by modulating NF-κB [10]. However, morin prevented the down-regulation of catalase by inhibiting NF-κB activation and thus helps to restore the cellular redox homeostasis in IND induced gastropathy [10]. Chattopadhyay et al. reported that curcumin (1,7-bis-[4-hydroxy-3-methoxyphenyl]-1,6-heptadiene-3,5-di one) (a major bioactive yellow pigment of Curcuma longa) dose-dependently exerts gastroprotective effect (primarily because of its antioxidant nature) by preventing peroxidase inactivation which generally got inactivated by indomethacin [16]. They showed that curcumin can effectively block indomethacin-induced lipid peroxidation and thiol depletion in gastric mucosa, which are considered as major antioxidative parameters [16]. In a different study, another free radical scavenger gallic acid (GA) has been shown to exert significant protection against NSAID-induced gastropathy by scavenging free radicals and blocking •OH-mediated oxidative damage [1]. It actually targets mitochondrial pathway of apoptosis by inhibiting mitochondrial oxidative stress thereby preventing mitochondrial protein carbonyl formation, lipid peroxidation, thiol depletion and restoring mitochondrial dehydrogenase activity. It also prevents NSAID-mediated activation of caspase-9 [1]. Now it is interesting to mention here that tea and tea derived polyphenols also contribute in this field as good protective agents. Antioxidant nature of the non-toxic tea polyphenol epigallocatechin gallate (EGCG) primarily contribute to its gastroprotective effect [111]. Similarly, the antioxidant activity of black tea (BT) and kombucha tea (KT) is the main factor behind their role as gastroprotective agents in the IND-induced gastropathy [17, 18]. In this way, there is an impressive list of naturally occurring antioxidants. They may be a complex mixture like Korean red ginseng extract or a simple compound like a catechol derivative, allylpyrocatechol (designated as APC, isolated from the leaves of the Indian medicinal plant Piper betle). In both the cases, their antioxidant action is primarily attributed to their antioxidant action [19, 20]. Besides, synthesized derivatives of these naturally occurring antioxidants also appear to be promising for different studies. Here the study of Pal et al. can be considered as a good instance [21]. They reported that synthesized tryptamine-gallic acid hybrid molecule (SEGA (3a)) prevents IND-induced gastric damage by inhibiting mitochondrial oxidative stress (MOS). SEGA scavenges mitochondrial superoxide anion (O2•−) and the released intra-mitochondrial free iron [21]. It blocks IND-induced mitochondrial protein carbonyl formation, lipid peroxidation, thiol depletion and inhibited down-regulation of bcl-2 and up-regulation of bax genes in gastric mucosa following IND exposure. It also prevented activation of caspase-9 and caspase-3 in IND-exposed gastric mucosa [21].

Besides these exogenous plant-derived or synthetic antioxidants supplementation of few endogenous compounds also provides excellent gastroprotection. In most of the cases again inherent antioxidant nature could be the key factor. But other unique properties could also be a contributor. Such compounds might be the CoQ10 (which can serve as a gastroprotective effect involving preservation of microvascular permeability, elevation of prostaglandin E2, improvement of redox status [22]), gastro-transmitter H2S or NO [23] or hormones. Published data showed that the hormone dehydroepiandrosterone (DHEA) exhibits protective effect against indomethacin-induced gastric ulcers by preventing lipid peroxidation and improving endogenous gastric antioxidant system [24]. Another hormone melatonin could also be considered here. Maity et al. showed that melatonin stops the NSAID induced development of...
mitochondrial oxidative stress and activation of mitochondrial pathway in the gastric mucosa [25]. Melatonin inhibits the downregulation of Bcl-2 and Bcl-xL. It also inhibits upregulation and mitochondrial translocation of Bak and the up-regulation of Bak. Likewise SEGA, it also reduces indomethacin-mediated activation of caspase-9 and caspase-3 by blocking the release of cytochrome c, thus preventing indomethacin-induced apoptosis [25].

**NSAIDs induced gastropathy: Inflammation and antiinflammatory compounds**

Besides oxidative stress, inflammation also plays a major role in the pathogenesis of NSAIDs induced gastropathy [5, 9, 11]. Oxidative stress is associated with pro-inflammatory cytokine production and, thus inflammation [1, 3]. Cytokines and chemokines orchestrate the general process of inflammation. Attraction and infiltration of leukocytes following pro-inflammatory cytokines and chemokines to the specific tissue site are often considered as hallmark phenomenon in the initiation of inflammation and pathogenesis of gastric mucosa [3, 9, 13, 26, 27]. IL-6, TNF-α, IL-1β, MCP-1, PGE2, and NO are mostly used biomarkers for gastric epithelial cell inflammation [7, 10]. Various adhesion molecules mediated leukocyte–endothelial cell interaction is also an early and important episode in the NSAIDs-induced gastropathy [28]. Here ICAM-1 and VCAM-1 play significant roles. Activated neutrophils persuade damage by occluding microvessels of gastric mucosa along with producing many pro-inflammatory and pro-oxidative enzymes like myeloperoxidase (MPO) and generating superoxide and other reactive oxidants [5, 11, 29]. Cumulatively these increase the oxidative load of gastric mucosa and damage the endothelial lining [9, 30-32]. We reported that the gastroprotective action of morin against IND-induced gastropathy is also attributed to its anti-inflammatory response, besides its potent antioxidant nature as mentioned above [10]. Morin reduced the IND-induced inflammation by inhibiting the NF-κB activation in IND-treated gastric mucosa. In fact, suppression of NF-κB plays the pivotal role here because NF-κB acts as the major transcriptional regulator of the pro-inflammatory genes expression. We showed that the morin directly suppresses the NF-κB activation by inactivating IKK and indirectly by modulating ROS, as ROS plays a very important role in NF-κB activation. This results in the transcriptional inhibition of a collection of inflammatory genes like iNOS, proinflammatory cytokines (TNF-α, IL-1β, IL-6), chemokine (MCP-1) and cell adhesion molecule (ICAM-1) in association with the decreased infiltration of neutrophils. A positive feedback loop plays an important role here. We suggest that IND induced increased cytokines activate NF-κB, which in turn produce the cytokines as mentioned elsewhere [10]. Now let’s see this phenomenon from another angle keeping NF-κB pathway aside which will help to prove the pharmacological acceptance of morin. Let’s focus on the cyclooxygenases (COXs), key players and drug targets in this pathophysiological condition. Here a very brief discussion on cyclooxygenase biology will be helpful for the readers. COXs are major enzymes in the eicosanoid biosynthesis pathway, converting arachidonic acid to PGH2, the precursor of all the prostaglandins, prostacyclins, and thromboxanes. COXs have two isoforms, COX-1 and COX-2. Both isoforms have two different enzymatic activities i.e., the cyclooxygenase activity (which catalyzes the formation of prostaglandin G2 from arachidonic acid by oxygenation), and the peroxidase activity (which catalyzes the formation of prostaglandin H2 from prostaglandin G2 by reduction). Nonsteroidal anti-inflammatory drugs (NSAIDs) are the competitive active site inhibitors of both the cyclooxygenase isoforms, COX-1 and COX-2, exist as homodimers. However, only one partner remains responsible at a time for substrate binding. NSAIDs inactivate the COX site (not the peroxidase) after binding at only one of the monomers of the COX dimer; resulting the shutdown of prostanoid formation [33]. COX-1 expression is marginally affected by inflammatory stimuli in most cases [34]. Interleukin-1β cytokine originates at a peripheral injury site and is unable to pass the blood-brain barrier [34]. It, therefore, remains in circulation and induces both COX-2 and membrane prostaglandin E synthase-1 (mPGES) activities in cells lining the barrier [35]. In the generation of the inflammatory response, prostaglandins play a key role. They contribute to the development of the cardinal signs of acute inflammation and their biosynthesis is increased significantly in inflamed tissue. During the acute inflammatory response, the pro-inflammatory properties of individual prostaglandins are more or less well established, their role in the resolution of inflammation is, however, more controversial [33, 34].

From the outcome of our study, we suggest that IND induces inflammation by producing pro-inflammatory cytokines. IL-1β is one of them. This cytokine in turn acts on the tissue and induces the production of inducible COX-2. However, in the entire process, the level of COX-1 remains unaltered. But paradoxically we observed that the PGE2 level decreased in the tissue in spite of increased COX-2 and unaltered COX-1 levels. Now, it is important to remember here that the induction of COX-2 does not signify its activation. IND-indirectly may up-regulate COX-2 but it effectively inhibits its biological activity by competitively inhibiting the arachidonic acid binding to the active site. That is true for both the isoforms. This phenomenon ultimately leads to decrease the level of PGE2. Here it is noteworthy to mention that the morin did not affect the prostaglandin biosynthesis. The result signifies that the morin is not impeding the pharmacological activity of the NSAID. Morin exerts its action by modulating different nodes lying
downstream of the NSAID activity, like the inflammation and oxidative stress [10]. In a different study, Yadav et al. pointed out that TNF-α upregulation was the primary event to initiate and induce gastric ulceration [7]. They also suggest that mitigation of TNF-α may offer a potential solution to IND-mediated gastropathy [7].

Presently the role of polymorphonuclear leucocytes (PMNs) in the pathogenesis of NSAIDs induced gastropathy is emerging as an important factor. Immuno-neutralisation of CD18, intercellular adhesion molecule 1 (ICAM-1), P-selectin or by rendering animals neutropenic via the intravenous administration of anti-PMN antiserum making them significantly resistant to the NSAID-induced gastric mucosal injuries [36, 37]. The findings of Morise et al. further strengthen the etiology. They observed an increase in gastric mucosal surface expression of P-selectin and ICAM-1 upon intragastric administration of IND. While administration of IND in CD18 or ICAM-1 or P-selectin deficient mice caused significant decreases in lesion scores compared with their C57BL/6 controls [1, 37].

Besides widely prescribed anti-ulcer drugs (often considered as non-safe), many naturally occurring plant-derived compounds are also found to be effective in combat against the NSAIDs-induced gastropathology. Curcumin was found to prevent indomethacin (IND)-induced gastropathy through the improvement of gastric microcirculation by attenuating the gastric inflammation (more specifically by attenuating the level of ICAM-1 and TNF-α) [38]. Another naturally derived compound arbutin was also found to modulate the levels of IL-6, IL-10 and TNF-α [39]. Very interestingly, Kinsey et al. found that Δ(9)-tetrahydrocannabinol (THC), the primary psychoactive component of marijuana, protects diclofenac-induced gastric inflammation and tissue damage at doses inadequate to cause common cannabinoid side effects [40]. An organosulfur constituent of garlic, 2-allyl cysteine (SAC), was also found to be effective against NSAIDs-induced gastric damages [41]. The researchers found that SAC was highly effective in a low dose through significant decreases in macrophage infiltration and IND-induced expressions of inflammatory mediators although a dose higher than ten micromoles showed an adverse effect. They, therefore, proposed that synthetic SAC can be a promising therapeutic agent against NSAID-induced gastropathy and could provide potent anti-inflammatory effects [41]. Amusingly, in the same year, Choi et al. established a synthetic SAC, PMK-S005, as a gastroprotective agent against acute gastric mucosal damage by NSAIDs. They showed that PMK-S005 pretreatment significantly attenuated NSAIDs-induced gastric damage by suppressing proinflammatory cytokines, down-regulating cPLA2, COX-2 and LTB4 expression [42]. On the other hand, another non-related synthetic compound SEGA (mentioned in the previous section) was found to inhibit IND-induced expression of ICAM-1, VCAM-1, IL-1β, MCP-1, and activation of NF-κB along with neutrophil infiltration [3].

Conclusions

Researchers all over the world are trying to solve the gastric problems related to NSAIDs by applying a wide range of approaches like modifying the specificity of NSAIDs, making them super selective to the “bad” COX, the COX-2, or trying to target the receptors of the proinflammatory prostanoids to minimizing the inflammatory responses. Also researchers are trying to develop potent H2S or NO releasing NSAIDs which are showing good effects over the traditional NSAIDs. NO and H2S are two endogenously generated important gaseous mediators which maintain gastric mucosal integrity and share many biological effects with prostaglandins. On the other hand, many groups are studying to develop various kinds of safe protective agents targeting directly the oxidative stress or inflammation in NSAIDs induced gastric damage. In most of the cases, they are natural herbal derivatives or in some cases they are synthesized ones. A number of such compounds hold immense potential but are under preliminary investigations. We strongly believe that the future detailed pharmacokinetic and pharmacodynamic studies on such compounds and genetic studies using genome-wide association and candidate gene approaches may further establish strong personalized medications against NSAIDs induced gastropathy.

Conflicting interests

The authors have declared that no competing interests exist.

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