The pathophysiology of non-steroidal anti-inflammatory drug (NSAID)-induced mucosal injuries in stomach and small intestine

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(Received 1 July, 2010; Accepted 13 July, 2010; Published online 26 February, 2011)

Non-steroidal anti-inflammatory drugs are the most commonly prescribed drugs for arthritis, inflammation, and cardiovascular protection. However, they cause gastrointestinal complications. The pathophysiology of these complications has mostly been ascribed to non-steroidal anti-inflammatory drugs' action on the cyclooxygenase inhibition and the subsequent prostaglandin deficiency. However, recent clinical demonstrated the prevalence of non-steroidal anti-inflammatory drugs-induced small intestinal mucosal injury is more often than previously expected. In this review, we discuss the defense mechanisms of stomach, and the pathophysiology of non-steroidal anti-inflammatory drugs-induced injury of stomach and small intestine, especially focused on non-steroidal anti-inflammatory drugs' action on mitochondria.

Key Words: **Non-steroidal anti-inflammatory drugs, gastrointestinal mucosal injury, mitochondria, lipid peroxidation, reactive oxygen species**

Non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin and indomethacin are the most commonly prescribed drugs for arthritis, inflammation, and cardiovascular protection. However, they cause gastrointestinal complications such as ulcers and erosions. The pathophysiology of these complications has mostly been ascribed to NSAID's action on the cyclooxygenase (COX) inhibition and the subsequent prostaglandin (PG) deficiency.[1] In 1970s and 1980s, under the concept of cytoprotection, extensive researches have revealed the role of PG in gastric mucosal defense system.[1]

Recent clinical researches shed a light on the NSAID-induced small intestinal mucosal injury. Capsule endoscopic examinations revealed that NSAID induced mucosal damages including erosions and ulcerations in small intestines more often than previously expected.[2] Although gastric mucosal injuries are considered to be associated with the gastric acid, these results suggested that NSAIDs induced mucosal injuries independently with the acid, since there is no acid-secreting cell in small intestines. Therefore, the treatment for NSAID-induced small intestinal mucosal injuries should include medications other than conventional acid-reducing agents such as histamine 2 receptor antagonists (H_2RAs) and proton pump inhibitors (PPIs).[3–6] The pathophysiological concept that will explain the NSAID-induced injuries of both stomach and small intestines is needed.

In this review, we discuss the defense mechanisms of stomach, and the pathophysiology of NSAID-induced injury of stomach and small intestine.

Gastric Mucosal Defense Mechanisms

Gastric mucosal injury occurs when the causative agents such as gastric acid and NSAIDs overwhelm the mucosal defense. The gastric defense mechanisms can be divided into three major components: pre-epithelial, epithelial, and post-epithelial defense mechanism (Table 1).[7,8]

Table 1. Gastric mucosal defense mechanisms

1. Pre-epithelial mechanisms
   - Mucous
   - Bicarbonates
   - Surface active phospholipids
2. Epithelial mechanisms
   - Tight junction complex
   - Restitution
   - Growth factors
   - Cell proliferation
3. Sub-epithelial mechanisms
   - Microcirculation of blood
   - Leukocytes

The pre-epithelial defense mechanism consists mainly of a mucous layer that contains mucous, bicarbonate and surface active phospholipids, and prevents epithelial cells from the contact with luminal noxious agents such as gastric acid. Mucous and bicarbonate are secreted by gastric epithelial cells. As a result, the pH at the surface of gastric mucosal epithelial cells normally is maintained in the neutral range when the pH at the gastric luminal surface reaches 1 to 2.

The surface epithelial cells are served as the second line of defense. Not only their tight junction complexes to limit the diffusion of hydrogen ions and their secretion of mucous and bicarbonate, but they migrate into a site of injuries to restore a damaged region (restitution).

Mucosal blood flow within the gastric submucosal layer comprises the post-epithelial defense mechanism. Blood flow provides an adequate supply of micronutrients and oxygen in order for epithelial cells to secrete mucous and bicarbonate. It also removes acid and other toxic metabolic by-products.

PGs play a key role in gastric epithelial defense by enhancing the pre-epithelial, epithelial, post-epithelial defense mechanisms: PGs regulate the secretion of bicarbonate and mucous, inhibit gastric acid secretion, and are important in maintaining epithelial cell restitution and mucosal blood flow.

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doi: 10.3164/jcbn.10-79

Proposed Mechanisms of NSAID-Induced Stomach Injury

The defense mechanisms of stomach against NSAID can be divided into 2 categories: PG-dependent mechanism and non-PG-dependent mechanism.

The pathophysiology of PG-dependent NSAID-induced stomach injury. Since PGs play a critical role in maintaining gastric mucosal defense system, the inhibition of COX leading to decreased mucosal PGs is considered as the most important in the pathogenesis of NSAID-induced gastric damage. Aspirin inhibits COX irreversibly, while other NSAIDs inhibit COX in a reversible, concentration-dependent manner.[8] Two COX isoforms were identified in early 1990s in mammalian cells, COX-1 and COX-2. COX-1 is constitutively expressed in most of tissues including stomach and responsible for maintaining gastric mucosal integrity at base line, whereas COX-2 participates in inflammation. Gastric injury was thus considered to be ascribed to gastric mucosal PG deficiency by COX-1 inhibition. In human stomach, little or no COX-2 protein and activity was demonstrated, while abundant COX-1 protein and activity was demonstrated.[9] Therefore, NSAIDs that selectively inhibit COX-2 were developed and applied in a clinical setting.

More recently, experimental data in animal demonstrated that for gastric ulceration to occur, both COX-1 and COX-2 must be inhibited.[10] The result challenges the concept that only COX-1 plays a housekeeping role in the stomach. Recent researches suggested that both COX-1 and COX-2 may play a role in PG synthesis and maintenance of gastric mucosal integrity, and that COX-2 plays a "back-up" role by alleviating PG deficiency which is induced by COX-1 inhibition.[1]

The pathophysiology of PG independent NSAID-induced stomach injury

The 'trapping' theory. The mechanism of NSAID-induced mucosal injury that is not dependent with systemic PG deficiency includes local injuries of these agents. Most NSAIDs are weak organic acids. In gastric juice, they are non-ionized and lipid soluble. These NSAIDs diffuse across gastric mucosal epithelial cell membranes into the cytoplasm, where pH is neutral. In neutral pH, NSAIDs are converted into the re-ionized and relatively lipophobic form. Therefore NSAIDs are trapped and accumulate within cells, leading to the cellular injury.[11] The mechanisms that NSAID induced local injury to the mucosal cell remain to be elucidated, but *in vitro* studies including ours proposed mitochondria are the primary target of NSAIDs, which is discussed in later sections.

This 'trapping' theory, however, may not be applied to small intestinal mucosa, where luminal pH is almost neutral. Considering the *in vitro* experimental results that NSAIDs are indeed absorbed into small intestinal cells in neural pH, we think the "trapping" may not be essential for inducing small intestinal injuries, but may accelerate the extent of injury by increasing the absorption of NSAIDs.

Mitochondria, lipid peroxidation, and apoptosis. A mitochondrion is a membrane-enclosed organelle with 0.5 to 10 micrometers range. In eukaryotic cells, mitochondria generate most of the cell's chemical energy supply of adenosine triphosphate (ATP). In addition to energy metabolism, the regulation of cell death has recently considered as a second major function of mitochondria.[12] Mitochondrial respiratory chain is the major source of reactive oxygen species (ROS), which are mainly generated at Complex I and III of the respiratory chain. More importantly, the mitochondrial respiratory chain is, at the same time, an important target for the damaging effects of ROS. ROS from mitochondria play an important role in the release of cytochrome *c* and other pro-apoptotic proteins, which can trigger caspase activation and apoptosis.[12]

Mitochondria are considered as the target intracellular organelle of absorbed NSAIDs. NSAIDs inhibit, or uncouple, oxidative phosphorylation to dissipate the mitochondrial transmembrane potential (MTP), leading to the liberation of cytochrome *c* from mitochondrial intermembranous space into cytosol and to the release of ROS such as superoxide ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2), thereby causing caspase 9 and caspase 3 activation and cellular lipid peroxidation, all resulting in cellular apoptosis.[13–16] The uncoupling of mitochondria also decreased the intracellular ATP concentration, leakage of Ca^{2+} out of mitochondria, cellular osmotic imbalance and a loss of control over intracellular junctions, resulting in increased permeability and subsequent mucosal damages.[17]

The Pathophysiology of NSAID-Induced Small Intestinal Injuries

The pathophysiology of NSAID-induced small intestinal injuries was less well understood than that of gastric injuries. For the pathophysiology of NSAID-induced small intestinal injuries, a three step hypothesis was proposed (Fig. 1).[18] Firstly, NSAIDs that were absorbed into the enterocytes inhibit the mitochondrial oxidative phosphorylation. Secondly, the inhibition of oxidative phosphorylation causes dysfunction of the tight intracellular junctions and increases the intestinal permeability.[2] Thirdly, through the mucosal barrier whose permeability was increased, the enterocytes are exposed to luminal contents, such as bile acids, hydroeolytic/proteolytic enzymes, pancreatic secretions, and finally intestinal bacteria, resulting in neutrophil chemotaxis with activation of neutrophils causing nonspecific inflammation and ulceration.[19]

The uncoupling of mitochondrial oxidative phosphoryalation. In these pathophysiological processes, the NSAID-induced inhibition of oxidative phosphorylation in mitochondria is considered as the main underlying mechanism.[2] The electron microscopic study of small intestine of rats treated with indomethacin revealed, within 1 h of treatment, swelling, vacuolation, ballooning and disruption of enterocyte mitochondria, which is consisted with the presence of the uncoupling of mitochondrial oxidative phosphorylation.[20]

The exact biochemical mechanism how NSAIDs inhibit, or

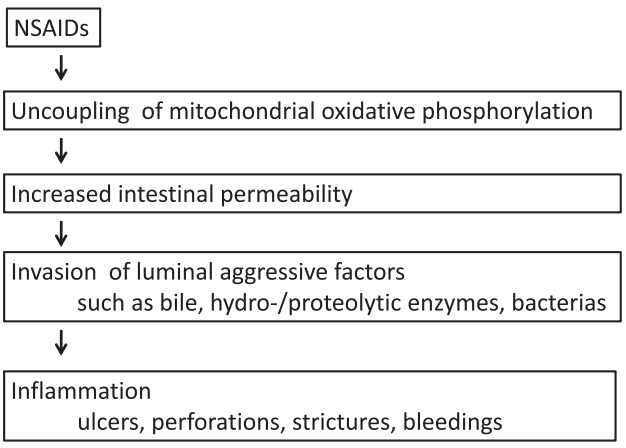


Fig. 1. The proposed pathophysiology of NSAID-induced small intesitinal mucosal injury. NSAIDs were absorbed into the enterocytes, and uncouples the mitochondrial oxidative phosphorylation. This uncoulping causes dysfunction of the tight intracellular junctions and increases the intestinal permeability. Through the mucosal barrier whose permeability was increased, the enterocytes is exposed to luminal aggressive contents such as bile acids, hydroeolytic/proteolytic enzymes, pancreatic secretions, and finally intestinal bacteria, resulting in neutrophil chemotaxis with activation of neutrophils causing nonspecific inflammation and ulcerations.

doi: 10.3164/jcbn.10-79

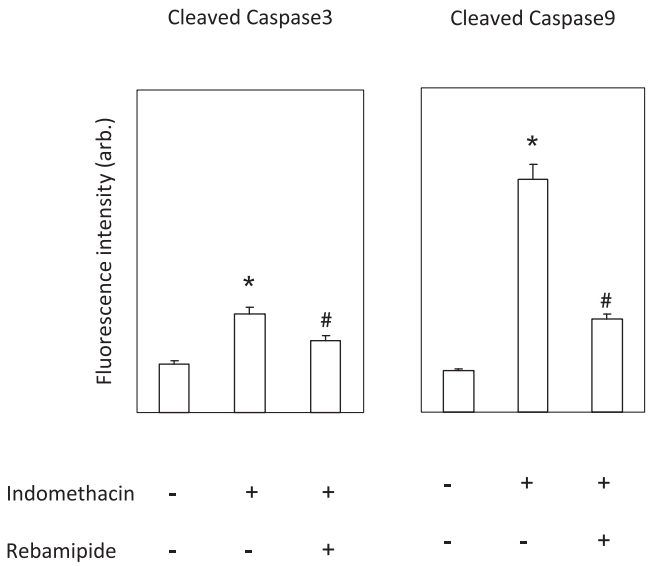
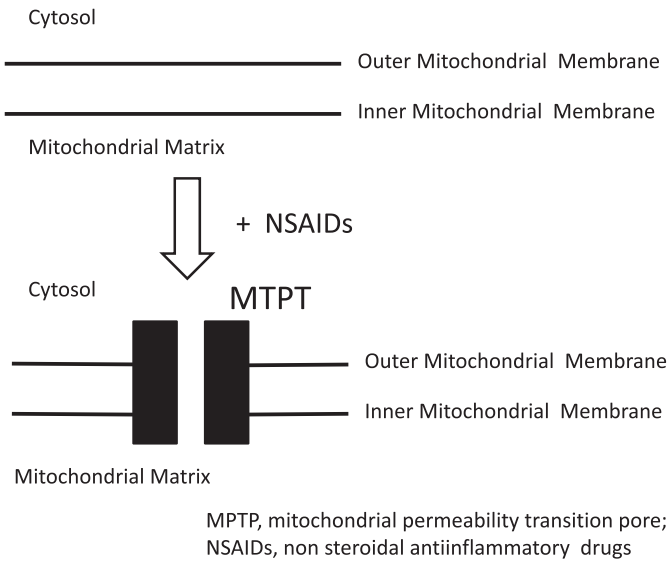


Fig. 2. Gastric epithelial RGM-1 cells were cultured with control medium, 1 mM indomethacin containing medium, and 1 mM indomethacin containing medium with 1 h 1 mM rebamipide pretreatment. Cells were immunohistochemically stained with monoclonal antibodies for caspase 3 and caspase 9. The fluorescence intensities of both caspase 3 and 9 were significantly increased in indomethacin-treated cells (*: $p<0.05$ vs control). However, the fluorescence intensities of cells pretreated with rebamipide were significantly reduced than those in cells treated with indomethacin alone (#: $p<0.05$ vs indomethacin alone).



MPTP, mitochondrial permeability transition pore;
NSAIDs, non steroidal antiinflammatory drugs

Fig. 3. The mitochondrial permeability transition pore. The MPTP is a protein pore that is induced in the membranes of mitochondria under certain pathological conditions or pharmacological agents. NSAIDs induce, probably through up-regulation of proapoptotic proteins and/or down regulation of antiapoptotic proteins of the Bcl-2 family, the translocation of proapoptotic Bax protein toward mitochondria, which further induces and opens MPTP. MPTP opening leads to a decrease of mitochondrial transmembrane potential, resulting in the inhibition of mitochondrial oxidative phosphorylation. Opening of MPTP also leads to cytochrome *c* realase into the cytosol, resulting in cellular apoptosis.

"uncouple", the mitochondrial oxidative phosphorylation is unclear. *In vitro* studies demonstrated that the uncoupling effect of NSAIDs is at least partly due to up-regulation of proapoptotic proteins and/or down regulation of antiapoptotic proteins of the Bcl-2 family,[21] followed by the induction of the mitochondrial permeability transition pore (MPTP).[22,23] The MPTP is a protein pore that is induced in the membranes of mitochondria under certain pathological conditions or pharmacological agents (Fig. 2). Some *in vitro* studies demonstrated that NSAIDs induce, through up-regulation of proapoptotic proteins and/or down regulation of antiapoptotic proteins of the Bcl-2 family, the translocation of proapoptotic Bax protein toward mitochondria, which further induces and opens MPTP.[21] MPTP opening leads to a decrease of mitochondrial transmembrane potential, resulting in the inhibition of mitochondrial oxidative phosphorylation. Opening of MPTP also leads to cytochrome *c* realase into the cytosol, resulting in cellular apoptosis.[21]

This process was also applied to gastric epithelial cells. Our study *in vitro* using gastric RGM1 cells also confirmed that indomethacin treatment induced a decrease of mitochondrial transmembrane potential and induced apoptotic protein activations (cleaved caspase 3 and 9), which was inhibited by an antiulcer drug rebamipide (Fig. 3).[16]

The immediate consequence of uncoupling of oxidative phosphorylation is a reduction of ATP production and subsequent leakage of Ca^{2+} out of mitochondria into cytosol.[24,25] The ATP deficiency and the accumulation of adenosine diphosphate (ADP) and adenosine monophosphate (AMP) stimulate glycolysis in enterocytes.[26] The maximally stimulated glycolysis induced the production of lactate rather than acelyl CoA, because pyruvate dehydrogenase activities are low in enterocytes.[17] The increased cytosolic Ca^{2+} activates Ca^{2+}-sentitive enzymes, proteases, endonucleases, and phospholipases, and reportedly potentiates cellular lipid peroxidation.[27]

The increased intestinal permeability. The mechanisms that the inhibition of oxidative phophorylation increases intestinal permeability are not well understood, but are explained as follows: the inhibition of oxidative phophorylation leads to intracellular ATP deficiency and leakage of calcium (Ca^{2+}) from mitochondria, both of which in turn causes increased cytosolic Ca^{2+}, increased production of ROS, disturbed sodium/potassium (Na^+/K^+) ratios with cellular osmotic imbalance, resulting in the dysfunction of the tight intracellular junctions and increased permeability.[2,17,19,28] In addition, NSAIDs-induced cellular apoptosis may involve in the increased permeability.[29]

The role of intestinal permeability in NSAID-induced small intestinal injury is extensively examined by Bjarnason and colleagues.[18,28] The increased intestinal permeability, which is assessed by measuring of urinary recovery of a variety of orally administered test probes such as ^{51}Cr-labeled ethylenediaminetetraacetate (^{51}Cr-EDTA), is induced within 12 h of NSAID administration in human, and it relates quantitatively to NSAID potency to inhibit COX, and is partly reversed by concomitant PG administration.[30] Interestingly, NSAIDs induced little or no increased permeability in stomach of human volunteers.[31,32] These results suggest the difference between the underlining mechanisms of NSAID-induced gastropathy and those of enteropathy.

The inflammation. Once the intestinal barrier has been broken, the sequence of events leading to inflammation is determined by the luminal aggressive factors. It is notable that the difference of aggravating factors between small intestine and stomach may explain the different biological responses and therefore the macroscopic lesions.[17] The aggravating factors of stomach are gastric acid and pepsin, whereas those of small intestine are bile acid, hydroeolytic/proteolytic enzymes, pancreatic secretions, and intestinal bacteria.

The important role of bile acid an aggravating factor of NSAID-induced small intestinal injury was suggested by the result of an *in vivo* study that bile duct ligation reduced the prevalence of the

lesions significantly.[33] Although the exact biological mechanisms were unclear, it is supposed that bile acid may further damage cellular membranes to increase intestinal permeability, or NSAIDs conjugated in bile may be converted into free, active form through the bacterial deconjugation in small intestine.

The role of bacteria as an aggravating factor of NSAID-induced small intestinal injury is suggested by the experimental results that NSAIDs induced very few intestinal lesions in germ-free animals and that pretreatment with antimicrobials reduced NSAID-induced small intestinal damages.[34–36]

The neutrophil infiltration is essential in the development of macroscopic lesion. The microbial invasion and the damaged mucosa are considered as the main neutrophil chemoattractant. Generated ROS from activated neutrophils results in the mucosal damage, the finding experimentally confirmed that the prevention of neutrophil infiltration by induction of neutropenia inhibited NSAID-induced macroscopic mucosal damage.[37]

The role of PG deficiency in NSAID-induced small intestinal injury. The role of PG deficiency in NSAID-induced small intestinal injury is poorly understood, and there is controversy regarding whether PG deficiency is considered as the main causes of NSAID-induced small intestinal injury. Some studies demonstrated the protective effects of PG on NSAID-induced small intestinal injuries in animal models[38–40] and the PG deficiency in small intestine is proposed as the main cause.[11,41]

However, recent experimental studies demonstrated that the PG deficient does not have a major role in the small intestine injuries.[42,43] Somasundram *et al.* studied in a rat model that was designed to allow dissociation of the effects and pathophysiological consequences of the 'topical' mitochondrial inhibition and the 'systemically' mediated COX inhibition.[44] They demonstrated that the treatment of parenteral aspirin decreased mucosal PGs without affecting mitochondria, cellular permeability and caused no inflammation, and that the treatment of dinitrophenol, an uncoupling agent, increased intestinal permeability and caused inflammation without affecting intestinal PG levels. They concluded that the deficient endogenous PG production is not sufficient to alter intestinal permeability in short term.[44]

Conclusion

This review is focused on the pathophysiology of NSAID-induced gastroenteropathy, especially on PG-independent, mitochondria-dependent small intestinal injury.

It seems that the framework of pathophysiology of NSAID-induced mucosal injury may differ in stomach and in small intestine. The relative physiological importance of PGs in maintaining mucosal defense system between stomach and small intestine may contribute to the experimental and clinical results of NSAID-induced mucosal injuries. In stomach, because of the essential role of PG, NSAID-induced mucosal injury can be explained by the PG deficiency, and the exogenous PG treatment had protective effects *in vitro* and *in vivo*. On the other hand, in small intestine, NSAID-induced mucosal injury cannot be explained, although controversy exists, by the PG deficiency alone. The difference in aggressive factors of mucosal epithelium may affect the pathophysiology of NSAID-induced mucosal injury: gastric acid in stomach and luminal bacteria in small intestine. The reduction of gastric acid secretion in stomach and the sterilization of bacteria in small intestine had a protective effect on NSAID-induced mucosal injuries, and not vice versa.

However, we emphasize the importance of the uncoupling of mitochondrial oxidative phosphorylation as a common first step in NSAID-induced mucosal injury both in stomach and in small intestine. We think a novel therapeutic approach against NSAID-induced gatrointestinal mucosal injuries should include agents that prevent the uncoupling oxidative phosphorylation of mitochondria in epithelial cells.

Abbreviations

NSAIDs	non-steroidal anti-inflammatory drugs
COX-1	cyclooxygenase-1
COX-2	cyclooxygenase-2
PG	Prostaglandin
H_2RAs	histamine 2 receptor antagonists
PPIs	proton pump inhibitors
ATP	adenosine triphosphate
ROS	reactive oxygen species
MTP	mitochondrial transmembrane potential
$O_2^{\bullet-}$	superoxide
H_2O_2	hydrogen peroxide
MPTP	mitochondrial permeability transition pore
AMP	adenosine monophosphate
ADP	adenosine diphosphate
EDTA	ethylenediaminetetraacetate

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