Review

Melatonin as an armament against non-steroidal anti-inflammatory drug (NSAID) induced gastric injury: An overview

Palash Kumar Pal[a], Bharati Bhattacharjee[a], Aindrila Chattopadhyay[b], Debasish Bandyopadhyay[a*]

[a]Oxidative Stress and Free Radical Biology Laboratory, Department of Physiology, University of Calcutta, 92, APC Road, Kolkata-700009
[b]Department of Physiology, Vidyasagar College,39, Sankar Ghosh Lane, Kolkata-700006
*Correspondence: debasish63@gmail.com; Tel: +91-9433072066

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ABSTRACT

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most widely prescribed medicines to treat numerous pathophysiological conditions clinically. However, growing evidence indicates the adverse effects of NSAIDs on the different vital organs, among which gastrointestinal (GI) tract seems to be the utmost target in most of the cases. NSAIDs promote over production of harmful reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the gastric mucosa. These toxic species cause microvascular damage, increasing intestinal permeability, leading to the development of gastric lesions including ulcerations. Several strategies have been proposed to reduce the side effects of NSAIDs on the GI tissue, but most of them have failed to achieve this goal. Identification of an appropriate therapeutic strategy is urgently required. It is our opinion that this novel strategy to target GI damage induced by NSAID should include both anti-inflammatory and antioxidant properties. Under such a circumstance melatonin probably is the best choice for this purpose. Melatonin is a broad spectrum antioxidant and anti-inflammatory molecule. Numerous studies have reported the protective role of melatonin against gastric tissue damages caused by NSAIDs in animals or clinically. However, the underlying molecular mechanisms are not fully clarified. Thus, the present review attempts to gather the available information on this topic to provide a clear understanding on the exact scenario of this aspect.

Keywords: NSAIDs, cyclooxygenase, gastrointestinal tract, oxidative stress, tissue damage, melatonin, antioxidant

1. INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are regularly prescribed globally for their anti-inflammatory, antipyretic and analgesic properties (1-3) to relieve pain and fever in rheumatic degenerative joint diseases and accidental injuries (4, 5). Despite of the beneficial effects, the uncontrolled application of NSAIDs has become a concerning due to their diverse harmful side effects on different organs, especially on the gastrointestinal (GI) tract (3). NSAIDs are reported to cause nephrotoxicity, hepatotoxicity and interrupted platelet functions. However, their serious toxicity frequently occurs in GI tract which could lead to death with high dose (6). The mechanisms of GI toxicity are complicated. For example, mercaptomethyl-imidazole (MMI) stimulates acid secretion in GI tract (7). In contrast, other NSAIDs block acid

secretion (8), therefore, to cause hypergastrinemia which triggers hyperplasia in gastric enterochromaffin like cells (9) and ultimately results in gastric lesions (10) and gastric carcinoma (11). To make the mechanisms even more complex is that these drugs are also known to interact with the cytochrome P-450 system and alter the metabolic pattern of several other drugs (12). Although numerous studies have been conducted to enlighten the underlying mechanisms as to how NSAIDs cause such detrimental pathophyiological conditions in the GI tract and what is the best protective remedy, a limited success has been achieved. The reason behind such failure might be due to the complex interplay of several organ specific factors and the functional characteristics of different NSAIDs that vary between individuals.

One of such major factors consistently found to be involved in various gastric pathology induced by NSAIDs is reactive oxygen species (ROS) or reactive nitrogen species (RNS) (13-15). NSAIDs, for example indomethacin, increase ROS production (16) and irreversibly inactivate peroxidases in the gastric mucosa (17). RNS also involve in gastric ulcerogenesis induced by NSAIDs in human as well as in various animal models (18). Gene expression analyses on gastric tissues in different animal models support such contention (18, 19). The overproduction of ROS/ RNS induced by NSAIDs may overwhelm the antioxidant defence system in diverse types of GI cells (20) and ultimately lead to cell death. Thus, the prime focus is to identify a potent antioxidant molecule with minimal or no toxicity to neutralize the ROS/RNS and protects the cells/tissues/organs from the deleterious effects of NSAIDs. These potential molecules should exhibit three properties of anti-secretory, antiulcer and antioxidant.

In this endeavor, discovery of melatonin (*N*-acetyl-5-methoxytryptamine) from bovine pineal gland (21) was a major breakthrough in a pharmacological aspect. In addition to its diverse physiological roles, melatonin is found to be a potent antioxidant and direct free radical scavenger (22-23) with minimal or no toxicity even at high pharmacological doses (24), It has drawn a great attention from researchers in context of protection against NSAIDs induced side effects. Considering its direct (as a free radical scavenger) and indirect (receptor dependent) actions of melatonin (22, 23) researchers have continuously tested this small molecule and tried to establish its therapeutic use in GI tract damage. Consequently, its beneficial effects on oxidative stress and drug-induced damage in gastric tissues have been observed (15, 25). The protective mechanism of melatonin on gastric injury associated with NSAIDs is not yet clearly understood and remains a topic of debating. It is important to gather the existing information to further understand its protective mechanisms on NSAIDs GI toxicity. Hence, this review summarizes the development of melatonin research from animal studies to human clinical trials. The information and discussion will definitely enhance our knowledge regarding the molecular and cellular mechanisms involving the development of gastric ulcers caused by NSAIDs and highlighting melatonin as its potentially primary therapeutic remedy.

2. NSAIDS PROMOTE DIVERSE PATHOPHYSIOLOGICAL CONDITIONS IN DIFFERENT ORGANS AND TISSUES.

NSAIDs including sulindac, sulindac sulfide, sulindac sulfone, aspirin, indomethacin, acemetacin, tolmetin, etodolac, ketorolac, and oxaprozin possess therapeutic benefits for pain relief, inhibition of inflammation, H_2O_2 detoxification (26,27,28), antipyretic and antithrombotic activities (28) and neuroprotection (29). However, they also caused detrimental side effects in different tissues/organs. For example, NSAIDs exhibit neurotoxic effects on the central nervous system (29), alter the night-time levels of melatonin and body temperature (30), cause cardiovascular and musculoskeletal complications (31), platelet dysfunction, convulsions (32), stroke, high blood pressure, incontinence in urinary functions, increase in fall risk, and even cancer in diverse organs, such as in the prostate (33), endometrium (34), oesophageal, head and neck (35). Long-term use of NSAIDs may also develop dementia,

depression, impaired cognitive function and other psychiatricdisorders (31). Among them, the most adverse effect of NSAIDs occurs on the GI tract from initial tissue damage and gastrointestinal bleeding to serious gastric inflammation and ulcers (15, 36). The differential side effects of NSAIDs on GI tract is due to the pharmacokinetics of these molecules and the local environments of GI. Biochemically NSAIDs contain monocarboxylic acid group which exhibits weak organic acidosis (pKa 3–5). At the acidic condition of GI tract, the NSAIDs are extracted (37). The carboxylic group, a basic structure of most of the NSAIDs, makes them to have high water solubility and detergent properties, thus they can interact with the surface membrane phospholipids and enter efficiently into the gastric cells causing unusual intracellular ion trapping (38).

3. MULTIPLE PATHWAYS ASSOCIATED WITH GASTRIC INJURIES INDUCED BY NSAIDS.

The GI injury caused by NSAIDs is a complicated and multistage process. During this process, the specific biochemical alterations and subcellular organelle damages are triggered by a series of tissue inflammatory reactions (39). The increased intestinal permeability (39), microvascular damage (40), gastric hypermotility (41), prostaglandin depletion (42), etc. have been implicated in the development of intestinal pathogenesis caused by NSAIDs.

NSAIDs cause intracellular energy depletion and cellular damages which finally increases intestinal permeability- a potent inducer of intestinal enteropathy (39). Patients receiving long term treatment of certain NSAIDs, particularly diclofenac, have frequently developed small intestinal enteropathy (38). The intestinal permeability is considered as one of the major therapeutic markers to evaluate the potential gastrointestinal damages caused by NSAIDs, especially in case of enteropathy (43).

Mucosal microvascular response is important for the mucosal defence since microvascular damage plays the initial and critical roles during the gastric ulcer development induced by NSAID (40). This contention is confirmed by the observation that the gastric ulcers caused by NSAIDs usually are present in the gastric antrum region where gastric mucosal blood flow level is lowest, possibly due to focal ischaemia (44). A significant correlation between decreasing mucosal blood flow and increasing injuries in the gastric antral region supports the argument mentioned above (44). Notably, the superficial erosions induced by NSAIDs occur mainly in the corpus region while the deep ulcers are in the antral region of the stomach (44).

A disruption of epithelial cell barrier in the gastric mucosa causes the topical injury with initial mucosal erosions while depletion of intracellular prostaglandin promotes the development of gastric and duodenal ulcers with use of NSAIDs (42). This assumption was based on the observation that inhibition of cyclooxygenase-1 (COX-1) by NSAID leads to the development of gastric hypermotility and microvascular disturbances in GI tract of rats with reduced levels of tissue prostaglandins (45).

Numerous studies have confirmed that gastric hypermotility is highly associated with the initiation and progression of gastric ulcers (41). The exact molecular mechanism of NSAIDs induced injury is currently unknown. It appears that high levels of NSAIDs are extracted in the GI tract and this temporarily restricts the blood supply to the gastric mucosa. The low blood supply and high tissue NSAIDs concentration synergistically promote microvascular damage and gastric hypermotility. If the process is prolonged, it enhances permeability of the mucosal layer and activity of myeloperoxidase, finally resulting in gastric lesions (41, 45). The unique pharmacokinetic and pharmacodynamic properties of NSAIDs add additional factors to promote the gastric inflammation and ulcer formation. The interactions between a particular NSAID and its pre-existing intracellular risk factors also contribute to the development of gastric ulcers (40). For example, a combination of indomethacin and bile is cytotoxic to the epithelial cells of the gastric mucosa. Making things worse is that NSAID-bile mixture

repeatedly passes GI tract via enterohepatic circulation, thereby, increasing the risk of gastric injury (46). In addition, neutrophils are also known to be potent inducers of NSAIDs mediated gastric enteropathy (47), although the underlying mechanism is far from being understood. The potential pathways are illustrated in figure 1.

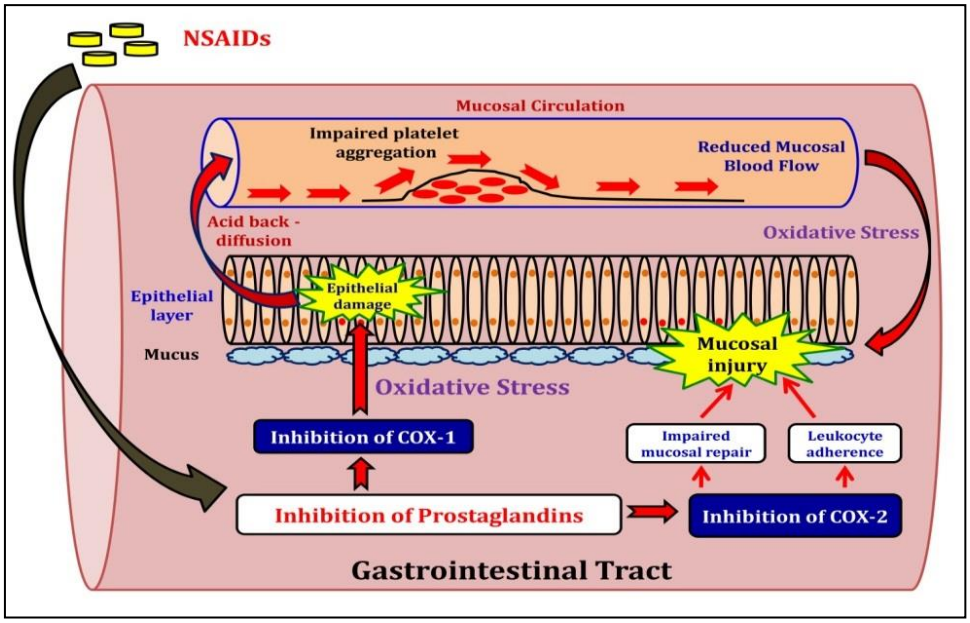

Figure 1: The illustration of potential pathways involved in NSAID-induced gastrointestinal damage.

4. NSAIDs AND PROSTAGLANDINS- A *VIS-A-VIS* INTERACTION.

The therapeutic actions of NSAIDs are principally based on antagonizing/blocking the synthesis of particular prostaglandins (PGs) through the inhibition of cyclooxygenases including cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) (3, 14, 15). It is well known that prostaglandin E2 (PGE2) and prostacyclin (PGI2) are required to maintain the integrity of gastric mucosa. The COX-1 and COX-2 are responsible for the production of prostaglandin endoperoxide (PGG2) and prostanoids from membrane bound arachidonic acid (AA) (48, 49). PGG2 is the precursor of PGE2 and PGI2 which are compelling vasodilators and are solely responsible for regulating mucosal defense and healing of the gastric tissues. Although the functions of them are correlated, the function of PGE2 is mainly mediated by four G-protein coupled receptors (GPCRs)- EP1, EP2, EP3 and EP4, using diverse signaling pathways while function of PGI2 is solely mediated through IP receptors (49). For example, when PGE2 mediates its signals through EP1 and EP2⁄EP4 receptors, it increases intra-cellular levels of calcium ion and cAMP, respectively, while cAMP level is reduced when the signal is transmitted via EP3 (49). The different effects of PGE2 may uncover the potential mechanism related to gastric injuries induced by NSAIDs. The syntheses of PGE2 and PGI2 are triggered by several events including feeding cues, levels of histamine or gastrin in the gastrointestinal tract. Under physiological condition, both PGE2 and PGI2 inhibit gastric acid secretion. Hence, PGs maintain the gastric mucosal integrity, promote healing and protect the gastric epithelium (50). NSAIDs significantly suppress prostaglandin synthesis and lead to systemic complications in the gastric mucosa. NSAIDs are weak acids and the ionization of NSAIDs in acidic gastric medium is inhibited, therefore, their absorption rate within the GI tract is high. In addition, the intracellular pH of gastric tissue lumen causes extensive ionization of NSAIDs;

thus, the NSAIDs molecules are trapped. The extremely high levels of NSAIDs in GI tract potentiate their anti-prostaglandin activities and result in severe mucosal injury (51, 52).

Being a bifunctional enzyme, cyclooxygenase (COX) displays both COX and peroxidase activities. COX can add two O_2 molecules to AA to generate PGG2 (an unstable cyclic hydroperoxide) which will be reduced by the peroxidase activity of COX to form PGH2 (an endoperoxide) (48). Finally, PGH2 is converted to biologically active and stable prostanoids (PGE2, and thromboxane A2) with several enzymatic processes. The peroxidase activity of COX is known to generate NAD^+ and $NADP^+$ radicals that lead to the production of O_2 (53). COX-1 is constitutively expressed in several tissues including the gastric mucosa and also regulates platelet hemostasis; whereas COX-2 is up-regulated only at the sites of any inflammation, tissue injury and tumorigenesis (54). COX-1 regulates the synthesis of prostaglandins and thromboxane A2 that primarily controls the mucosal defense in GI tract and numerous other physiological aspects. On the other hand, COX-2 controls the production of PGs that are associated with pain and inflammation (3).

Evidence from animal studies suggested that COX-1 dependent PGE2 depletion decreased blood flow in the gastric tissue while COX-2 inhibition induced leucocyte adherence (55). The inhibition of both COX-1 and COX-2 by NSAIDs causes severe gastric lesions (55, 56). At physiological condition, COX-1 is profoundly expressed in the gastric mucosa while COX-2 expression is low.However, COX-2 levels will be significantly up-regulated when COX-1 expression is inhibited during tissue injury or in pre-existing ulcers. COX-2 derived prostaglandins are the prime regulators for the healing of gastric ulcer, while during their shortage the COX-1 derived prostaglandins will take over and play the similar protective roles (57).

The anti-inflammatory, antipyretic and analgesic functions of most NSAIDS are mediated through the inhibition of COX-2 and some also inhibit the activity of COX1. Inhibition of both enzymes inevitably promotes gastric toxicity (3). Noteworthy, different NSAIDs possess varied specificity for COX-1 and/or COX-2 (58). For example, indomethacin is a non-selective COX inhibitor. Thus, the dose required to suppress COX-2 activity at an inflammatory site will also suppress COX-1. On the contrary, NSAIDs, such as naburnetone and etodolac, are highly selective COX-2 inhibitors and therefore, the ulcerogenic potentiality of these drugs is considerably reduced due to their low specificity toward COX 1 (58, 59). Even though these NSAIDs can selectively inhibit COX-2, for example coxibs, they still have the potential to induce the gastric ulceration due to inhibition of the production of cyto-protective prostaglandins (3). It was reported that the inhibition of platelet COX-1 expression by non-specific NSAIDs decreases thromboxane production and increases bleeding tendency in the concerned tissue. This is a major cause of gastric bleeding complications related to NSAIDs (such as piroxicam) (40, 60, 61). Thus, no matter, whether it is selective or non-specific inhibition of either COX-1 or COX-2 by NSAIDs, it will cause gastric mucosal injury by different degrees (56, 62). For example, expressions of both COX-1 and COX-2 are found to be up-regulated at gastric ulcer margins in humans, whereas under similar patho-physiological condition only COX-2 is up-regulated in rats (63, 64). Selective inhibition of COX-1 caused gastric hypermotility and increased microvascular complications in rat (45). Inhibition of both COX-1 and COX-2 caused more damage during ulcer healing than that treated with selective inhibitor of COX-2 in mice. Interestingly, no obvious effect on ulcer healing was found following selective inhibition of COX-1 (57). In contrary, COX-1 derived PGE2 is involved in early stage protection on gastric injury (65). The NSAIDs with both selective and non-selective COX inhibitory properties, such as naproxen, aspirin and celecoxib, delay healing of gastric ulcers in humans and animals (57, 66,67). In order to understand the underlying molecular interaction between NSAIDs and COX enzymes, aspirin is a better example since it has been investigated thoroughly. In brief, aspirin covalently binds and acetylates the serine residue (Ser

530) present in the active site of the COX enzymes. Such binding to COX-1 promotes conformational change of the enzyme structure to interrupt formation of oxidizing arachidonic acid, thereby inhibiting the activity of OX-1 (68). However, binding of aspirin to serine residue (Ser 516) in COX-2 is unable to inhibit formation of oxidizing arachidonic acid, thus it continues to produce 15-R-hydroxyeicosatetraenoic acid (15-R-HETE). 15-R-HETE, then is further metabolized by lipooxygenases to a potent neutrophil inhibitor, known as 15-epi-Lipoxin A4 (15 epi-LXA4) (68).

5. OTHER FACTORS IN GASTRIC DAMAGES INDUCED BY NSAIDs.

Although the gastrointestinal injury induced by NSAIDs is primarily due to blocking of prostaglandin synthesis through inhibition of the COX enzymes, this is not the only underlying mechanism but other prostaglandin independent pathways are also involved (40). Many autacoids along with prostaglandins co-orchestrate to maintain the integrity of the gastric mucosa and promote ulcer healing. These autacoids include nitric oxide and hydrogen sulphide (H_2S) as the gaseous mediators, neuropeptides (such as calcitonin gene related peptide [CGRP] and substance P), hormones (such as melatonin and gastrin), matrix metalloproteinases, growth factors, polyamines and stress proteins (40). As mentioned above, NSAIDs can accumulate in the gastric epithelial cell due to 'ion trapping'. This accumulation interferes in mitochondrial oxidative phosphorylation and finally disrupts the electron transport chain. Therefore, this activity depletes intracellular level of ATP, induces Ca^{2+} toxicity and eventually enhances the generation of ROS. Overproduction of ROS is detrimental to cell since they oxidize proteins, lipids or nucleic acids and impede important intracellular signalling pathways, even lead to cellular necrosis and apoptosis (69). NSAIDs, such as indomethacin and aspirin, disrupt the hydrophobic barrier of the epithelial lining by establishing chemical association with extracellular phospholipids lying on and within gastric mucosa (70-72). Acid back-diffusion via damaged gastric mucosa plays a key role in promoting superficial lesions to serious mucosal ulcers (73-74). Induction of different growth factors, various cytokines (IL-1β and IL-6) and hormones (such as gastrin) at the ulcer margins by PGE2 is also involved in the abatement of complex pathogenesis induced by NSAIDs in the GI tract (75). Therefore, maintenance of an appropriate level of cyto-protective PGE2 in the gastric mucosa is very important. Depletion of this cyto-protective prostaglandin causes gastric mucosal injury. Leukocyte accumulation induced by NSAID is another culprit in the development of gastric mucosal injury either by enhancement of tissue ROS and proteases, or by reduction of gastric blood flow through formation of capillary obstacle (76).

6. EFFECTS OF NSAIDS ON FREE RADICAL GENERATION IN GASTRIC TISSUES.

Oxidative stress is a physio-pathological condition with excessive production of ROS which disrupts intracellular antioxidant defense system (25). Re-establishment of normal cellular oxidative balance depends on the adaptability and repair-replacement capability of the cells/tissues (77). The role of ROS in the gastric ulcerations induced by NSAIDs has been well documented in animal studies and also in clinical trials (78, 79). Numerous NSAIDs, such as aspirin, indomethacin, ibuprofen, etc., are known to induce oxidative stress in the gastric tissues by inhibiting prostaglandin synthesis, an essential gastro-protective molecule (80). NSAIDs also inhibit cell proliferation and deplete cellular energy supply, thus leading to increased levels of H_2O_2 and hydroxyl radical ($^{\bullet}OH$) in the gastric mucosa which results in oxidative mucosal damage (7, 78). A gene expression study in the rat stomach has also confirmed this observation (19). For example, indomethacin treatment irreversibly inactivated gastric peroxidase in the rat gastric mucosa, and caused oxidative stress in this tissue (17). Piroxicam administration

elevated the level of •OH in the gastric tissue of rats and caused depletion of antioxidants, lipid peroxidation, protein oxidation and gastric ulceration. (61). NSAIDs also target mitochondria by disrupting its trans-membrane potential and increasing permeability transition pore with cytochrome c release (81). Mitochondrial damage inevitably promotes ROS production, induces caspase cascade and membrane lipid peroxidation and cellular apoptosis (39). Consequently, mitochondrial damage, in turn, increases intestinal permeability, mucosal erosion and intestinal injuries as well (39).

In addition to ROS, the RNS is also involved in NSAID induced gastric ulcerogenesis (18). NSAIDs enhance the activity of endothelin-converting enzyme-1 (ECE-1) to increase intracellular endothelin-1 (ET-1) which suppresses the expression of cNOS to reduce endothelial nitric oxide and ultimately distorts mucosal integrity (18). Indomethacin increased serum levels of NO by up-regulation of iNOS expression in activated circulating neutrophils while at the same time g astric NO levels are decreased since the gastric mucosal cNOS expression was down-regulated (82). Interestingly, up-regulation of iNOS mRNA expression is reported to involve in gastric ulcerogenesis in arthritic rats (83). An increase in gastric tissue iNOS activity is also observed in human volunteers when treated with ibuprofen (84). The mechanisms by how that NOS regulates the gastric physiopathology were reported by Souza *et al*. (85-86). For example, indomethacin caused significantly less gastric mucosal damage in animals pre-treated with either non-specific NOS inhibitor (NG-nitro-L-arginine methyl ester) or specific iNOS inhibitor (85, 86). The levels of both iNOS and eNOS derived NO are increased in NSAID-induced gastric ulcer healing process indicating that NO participates in the healing process under pathological conditions rather than in the normal healing scenario (57).

7. IMPACTS OF NSAIDs ON CELLULAR STRESS RESPONSIVE PROTEINS AND APOPTOSIS.

A number of *in vitro* and *in vivo* studies have shown the capability of NSAIDs in up-regulating several stress responsive proteins, such as heat shock protein 72 (HSP-72), glucose-regulated protein 78 (GRP-78), and HO-1 (also known as HSP-32) (87). Stress responsive proteins play an important role as potent cyto-protectors of gastric mucosa from NSAID-induced cellular apoptosis, particularly when synthesis of prostaglandin is hampered by NSAIDs. Notably, resistance to NSAID-induced gastric erosions and ulcers was reported in transgenic mice with a particular phenotype which was over expression of human HSP27 (88). Moreover, NSAIDs (such as sodium diclofenac, flurbiprofen, zaltoprofen, etodolac, etc.) are reported to induce DNA fragmentation associated to cellular apoptosis and enhances COX-2 mRNA expression in isolated AGS cells derived from human gastric epithelium (89).

8. DEVELOPMENT OF PREVENTIVE STRATEGIES.

Considering multiple side effects of NSAIDs on gastric tissue, it is necessary to develop their alternatives with no or less side effects to treat inflammation. To achieve this goal, a better understanding of the normal physiology and gastric mucosal defence mechanisms is essential (90). Although many attempts have been made to try overcoming the pro-inflammatory and topical irritant properties of NSAIDs, most of them have failed to reduce gastric ulcerogenesis, tissue perforation and bleeding complications (91). The major cause behind such failure was that the structural modifications for the new NSAIDs indeed improved their topical irritancy but were unable to modify their inhibitory effect on the synthesis of gastric prostaglandins. Some of the attempts are discussed herein. Firstly, incorporation of a nitric oxide (NO) generating moiety into the NSAID molecule was desired to suppress its side effects. Since, NO is well known for its potent vasodilation property and neutrophil associated functions, the

design was predicted to counteract the harmful effects of COX suppression in the gastric microcirculation and to reduce mucosal injuries (92). The rational to select NO as a tag to NSAIDs was based on the notion that inhibition of NO synthesis might associate with NSAIDs induced gastric damages (93). Moreover, NO and NO donors were found to reduce the clinical severity of gastric injuries in experimental animal models (68, 94). It was observed that rats with pre-existing colitis treated with NO-NSAIDs on daily basis, caused serious ulceration in the small intestine, leading to gastric perforation and ultimately death (95). In addition, the derivatives of NO-NSAIDs also possessed adverse effects since they shared same property to suppress gastric prostaglandin synthesis as their parent NSAIDs (95). Secondly, co-administration of a prostaglandin analogue and NSAIDs was suggested as an alternate strategy in reducing ulcerogenic characteristics. Thus, administration of PGE1 analogue**,** misoprostol, was found to markedly decrease the ulcerogenic incidences caused by any NSAID following long term treatment (96). However, other severe side effects, such as diarrhea, as well as the cost effectiveness issues impeded this strategy (97). Latter discovery of two isoforms (COX-1 and 2) of cyclooxygenase has restricted in designing novel anti-inflammatory drugs/agents that were assumed to spare the gastrointestinal tract.

In addition, several other strategies were also used in an attempt to reduce NASIDs associated side effects. These included enteric coating technology to reduce gastric absorption, parenteral administration, designing several proto-drugs that requires metabolism in the hepatic cells for their desired effects on cyclooxygenase activity, synthesis of basic molecules instead of acidic properties, co-administration of exogenous prostaglandins or acid secretion inhibitors or COX-2 inhibitors (52,98). However, none of these attempts were successful due to their adverse reactions and severe ulcerogenic properties again in the GI tract (51). Thus, chronic use of NSAIDs was not recommended by the Beers Criteria of The American Geriatric Society in 2015.due to their high risk of gastric bleeding. Interestingly, administration of synthesized cysteamine (a well-accepted antioxidant) tagged amide derivatives of some NSAIDs, such as diclofenac acid, tolfenamic acid, ibuprofen and indomethacin, was found to significantly reduce gastric ulcerogenesis in animal models (13). Such observation requires further careful investigation before its clinical use. It seems that if NSAIDs possess both anti-inflammatory and antioxidant activities along with reduced acidic property, the novel NSAIDs, will potentiate their anti-inflammatory action while they will also cause none or less side effects in the GI tract (13).

9. MELATONIN- A MULTI TASKING MOLECULE IN THE GASTROINTESTINAL TRACT.

Melatonin (*N*-acetyl-5-methoxytryptamine) is not only synthesized in the pineal gland, but its production has also been identified in several extra-pineal tissues/organs in vertebrates (99), including the gastrointestinal tract (100). Presence of melatonin in the GI tract was first reported in rat (101). Subsequently, melatonin was immuno-histologically detected in the enterochromaffin cells (EC) of the gastric mucosa in rat (102-105) and its presence in the gastrointestinal tract was confirmed by radioimmunoassay (RIA) and high performance liquid chromatography (HPLC) (106-107). Using autoradiography, the maximum binding of melatonin was detected in the mucosa and intestinal villi (104, 108). At the sub-cellular level, the highest melatonin binding was found in the nuclear fraction, followed by the microsomal, mitochondrial, and cytosolic fractions (109). Notably, arylalkylamine-*N*-acetyltransferase (AANAT) as the rate-limiting enzyme in melatonin biosynthesis was identified in GI tract (110). Not only melatonin but melatonin receptors are also found abundant in the GI tract (111). As a signal molecule, melatonin regulates seasonal and circadian rhythms, reproduction, day/night activity, sleep behaviour and many other physiological events in vertebrates (99). In

addition, melatonin also participates in the protection of gastric tissues from oxidative and inflammatory injuries (79, 112).

10. RELEVANCE OF MELATONIN RECEPTORS IN GASTRIC TISSUE.

Melatonin receptors and/or binding sites have been identified in the GI tract of vertebrates including human (108,113, 111). The GI derived melatonin also releases into the peripheral circulation (79,114, 115) All of these clearly suggest the paracrine function of GI melatonin. It is widely accepted that most of the physiological effects of melatonin in the GI tract are mediated by activation of its membrane receptors. Two melatonin membrane receptors have been reported, that is MT1 and MT2. These receptors belong to the G-protein receptor family. A third receptor for melatonin, MT3, is also under consideration though it is classified to be a quinine reductase 2 (116). Current knowledge indicates that melatonin is also likely to act through its nuclear receptors (or binding sites) which belong to the RZR/ROR orphan receptor family including three subtypes (α, β, γ) and four splicing variants of the α subtype (117). Melatonin acts on these specific receptors to exert its physiological and pharmacological effects on the target cell/tissue/organ. For example, melatonin acts on MT1 receptor to stimulate G proteins (Giα2, Giα3, Gαq) and to inhibit the cAMP signalling pathways (118); whereas its action on MT2 receptors is associated with phosphoinositide signal transduction pathways and inhibits the adenylyl cyclase and guanylyl cyclase pathways (119).

11. MELATONIN AS AN ANTIOXIDANT IN GASTRIC TISSUE.

Melatonin, being an amphiphilic molecule, can easily pass through any biological membrane, thus possesses a free access to any cell/tissue/organ (120). Such an advantage renders this indolamine with diverse antioxidant properties that not only protects cells from oxidative damage induced by ROS but also from RNS (120-122). Melatonin is a broad spectrum antioxidant with a direct and indirect free radical scavenging property (121-122). It is especially effective in scavenging the most reactive and harmful •OH, a property of melatonin first reported by Tan *et al.* (22). Despite of the notable presence of various other endogenous antioxidants, melatonin is found to be unique due to its diverse actions on different cells/tissues. It not only directly scavenges free radicals (22), but also reduces the production of reactive species by inhibiting the activities and/or expression of pro-oxidative enzymes. Thus, evaluation of the functional characterization of melatonin and its physiological relevance in regulation of oxidative status in diverse cells/organs, especially gastrointestinal tract (61, 39, 79, 112) became the prime focus of the researchers from the last decade (120, 122).

Several lines of evidence were in favour of melatonin acting as a potent detoxifier of the highly toxic peroxynitrite anion, hydroxyl-, peroxynitrite-, and peroxyl radicals and singlet oxygen (23, 122-126) to protect against oxidative membrane damage (125) under diverse gastric pathophysiological conditions (39, 125, 127). Available data have unequivocally demonstrated the antioxidant capacity of melatonin in GI tract of vertebrates (125, 127-130). Not only melatonin but its metabolites are also the potent antioxidants (127, 131) to scavenge highly toxic hydroxyl radical, peroxynitrite anion as well as peroxyl radical and to quench singlet oxygen (124). They cooperate with other intracellular antioxidants to maintain membrane integrity and protect the cells from the deleterious ROS (127). Basically, under oxidative stress, melatonin executes its antioxidant action on the target cells via two pathways One is receptor-independent, that is, melatonin directly scavenges harmful free radicals. The other is its receptor dependent and indirect pathway. In this pathway, melatonin regulates the levels/activities of numerous intracellular enzymatic or non-enzymatic antioxidants (14, 23, 132). It was reported that melatonin up-regulated the levels of mRNA and activities of different endogenous antioxidant enzymes including SOD, CAT, GPx, GR, glucose-6-phosphate

dehydrogenase (G6PD) and gamma-glutamylcycteine synthase under oxidative stress (23, 122, 133).

Currently, the attention has been given to the effects of melatonin on the mitochondrial physiology. Melatonin is able to reduce electron leakage from the mitochondrial electron transport chain (134), increases mitochondrial respiration and ATP synthesis by enhancement of activities of complex I and IV (134-136). Notably, mitochondrial matrix is the primary site for free radical generation during electron transfer to molecular oxygen. On the other hand, melatonin inhibits NOS activity, ultimately reducing NO production in the cell/tissue (126). All these activities render melatonin to effectively prevent proteins, lipids, as well as DNA from oxidative damage (122, 137-138). Therefore, due to the potent antioxidant and free radical scavenging properties, melatonin is suggested as a potential therapeutic agent in the inhibition and healing of gastric injury induced by any sort of oxidative damage, particularly induced by NSAIDs (139-140).

12. MELATONIN- A POTENT THERAPEUTIC AGENT AGAINST NSAIDs-INDUCED GASTRIC INJURY.

It was well documented that melatonin effectively protected against gastric injuries caused by a variety of experimentally induced stress in the GI tract (79,112, 141-142) and this suggested the potentially beneficial effects of melatonin against NSAID-induced oxidative stress and gastric tissue injury. Since melatonin treatment could suppress ROS in circulatory system and prevent neutrophil accumulation locally (79, 112), it clearly indicates its protective potential against any pathophysiological condition in the GI tract. It was observed that, prostaglandins enhanced nocturnal melatonin synthesis in the gastric tissues (79) while NSAIDs, such as aspirin and ibuprofen, inhibited the production of PGs and reduce the synthesis of gastric melatonin; thus, an important factor of GI tract damage induced by NSAIDs may be related to the reduced melatonin levels in the GI tract of human (143).

12.1. Melatonin increases mucosal blood flow and promotes ulcer healing:

Melatonin has been reported to exhibit dose-dependently protective effects on indomethacin as well as piroxicam- induced gastric damages in rat (61). Likewise, aspirin induced mucosal damages in the GI tract of human is also significantly reduced following oral administration of melatonin as well as its precursor L-Tryptophan (144-145). Interestingly, an increase in circulating levels of melatonin was observed in aspirin-induced acute GI injuries and this indicated that the synthesis of melatonin in the gastric tissue was induced and probably the tissues try to combat against the injury (144). The same study also depicted that exogenous melatonin and L-tryptophan increased the levels of mRNA expression of MT_2 and two major enzymes of the melatonin biosynthetic pathway,AANAT and hydroxyindole-O-methyltransferase (HIOMT) along with an enhanced blood flow in the gastric mucosa. These results suggest the potential ulcer healing property of melatonin in the gastric tissue (144). Melatonin and L-Tryptophan are reported to increase the mucosal blood flow and release of NO in the lumen of GI tract in rats that were under indomethacin or NOS (L-NAME) administration (16, 146).

12.2. Melatonin scavenges free radicals and inhibits development of gastric ulcer.

Melatonin acting as a direct as well as indirect antioxidant was observed to improve the intestinal permeability and restore mucosal architecture at ultrastructural level which were distorted by diclofenac treatment (147-148). The mechanism might involve in altering the ion channels (148) to re-establish membrane potential and restore normal energy metabolism in the mitochondria and thus, preventing cellular death (150). Additional studies clearly demonstrated the capability of melatonin in preventing ROS mediated gastric ulceration

induced by NSAIDs, such as piroxicam (61). This was achieved by the melatonin's capacity to restore the activities of gastric peroxidise, superoxide dismutase, catalase and to lower tissue levels of ·OH, thus providing direct evidence regarding antioxidant and free radical scavenging properties of melatonin against gastric injuries induced by NSAIDs (61). Mechanistic exploration indicated that protective effects of melatonin on indomethacin-induced gastric injury and its healing process were primarily mediated by its MT_2 receptors (151). MT2 receptor activation increases MMP-2 expression, attenuates the MMP-9 activity and TIMP-2 expression and finally lowers the ROS in the gastric tissue (152-153). Numerous studies in mammals, have shown that melatonin dose-dependently inhibits secretion of gastric acid, reduces neutrophil infiltration and cytotoxicity by, enhances gastric mucosal blood flow at ulcer bed, induces secretion of bicarbonate in the duodenum, stimulates diverse intracellular defence systems associated with the cycloxygenase-prostaglandins and NOS-nitric oxide as well as CGRP (145,154-155) (Fig. 2). Finally, potent anti-apoptotic action of melatonin should also be added to its protective effects on the gastric mucosal injury induced by NSAIDs (139).

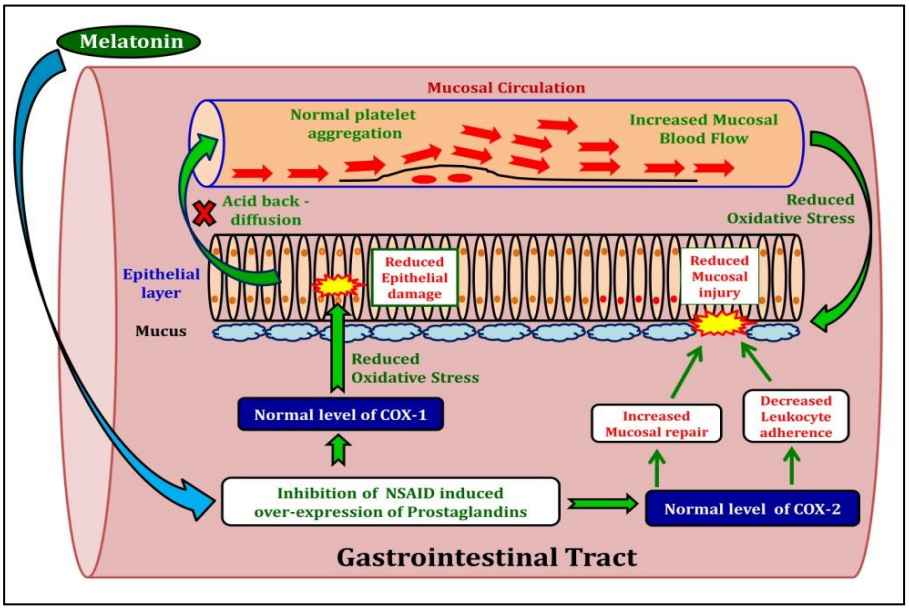

Figure 2: The illustration of the potentially protective roles of melatonin against NSAID-induced gastrointestinal damage.

12.3. Melatonin as an alternative to NSAIDs.

The beneficial roles of melatonin against NSAID-induced gastric injury have been listed in the table 1. In addition to the protective effects of melatonin on gastric injury induced by the NSAIDs, melatonin *per se* can also decrease inflammatory pain, by inhibiting NO generation and regulating the signalling pathways of NO-cyclic GMP (156). The analgesic action of melatonin involves several components and diverse pathways including β-endorphins, GABA receptor, opioid l-receptors and the nitric oxide (NO)- arginine pathway (157-158). Interestingly, due to steric and electronic complementarity, melatonin can directly bind to the active site of COX-1 and COX-2 acting as a natural inhibitor of COX to suppress the inflammatory reaction (158-159). Its application not only reduces the GI tract injury induced by the NSAIDs but also improves the anti-inflammatory effects of NASID.

Table 1: Beneficial roles of melatonin against NSAID-induced gastric injury.

NSAID	Adverse effects on the GI tract	Affected cellular agents	Effect of melatonin	Applied doses of melatonin	References
Indomethacin	(i) Disrupts epithelial lining (ii) Develops gastric ulcer	Inhibits synthesis of prostaglandins (PGs)	(i) Enhances ulcer healing process (ii) Increases mucosal cell proliferation (iii) Promotes mucosal repair	20 mg/kg	17, 58, 72, 147-148, 160
Piroxicam	(i) Increases gastric bleeding complications (ii)Develops gastric ulcer	Inhibits gastric COX-1 level	(i) Restores gastric COX-1 level (ii) Decreases gastric ulceration	60 mg/kg	40, 60-61
Naproxen	Delays gastric ulcer healing	Acts as selective and non-selective inhibitors of COX	No data available	No data available	57, 67
Aspirin	Delays gastric ulcer healing	(i) Acts as selective and non-selective inhibitors of COX (ii) Decreases synthesis of gastric melatonin	Enhances gastric ulcer healing process	2.5-10 mg/kg	57, 67, 70-72, 79
Celecoxib	Delays gastric ulcer healing	Acts as selective and non-selective inhibitors of COX	No data available	No data available	57, 66, 67
Diclofenac	Increases mucosal permeability	(i) Damages DNA (ii) Increases gastric COX-2 expression	(i) Decreases intestinal injuries (ii) Restores intestinal permeability	10 mg/kg	39, 89, 149
Ibuprofen	Increases gastric disease severity	(i) Inhibits PGs (ii) Reduces gastric melatonin synthesis	No data available	No data available	144

13. FUTURE PERSPECTIVES.

There is no doubt that unregulated application of NSAIDs is a potent threat to cause diverse gastrointestinal complications leading to gastric bleeding, ulceration and even causing death of the individual. Reduced levels of mucosal prostaglandins and increased ROS seem to convey the most adverse effects of NSAIDs in the GI tissues. The low levels of PGs also cause low melatonin production in the gastric tissue which leads to the vicious cycle of oxidative stress and gastric injury in GI tract. Therefore, elaborate studies regarding the structural

complementarity of a potent antioxidant- melatonin with prostaglandins related enzymes (COX-1 and 2) may evolve new strategy to overcome the side effects of NSAIDs in the gastric tissues. Numerous NSAIDs and existence of variety of functional pathways of them make the situation much more complicated and challenging to develop novel therapeutic agent with no or least gastric toxicity. It is our opinion that the novel NSAIDs must be re-designed in such a way that they possess both anti-inflammatory and antioxidant activity along with ability to reduce acid secretion. Overwhelming evidences on the multi-functional property and numerous beneficial characteristics of melatonin in animal studies and clinical trials clearly indicated this indolamine as an effective alternative against the gastric toxicity induced by different NSAIDs, although further careful investigations are required to reveal the exact underlying molecular mechanism(s). Here, we suggest that melatonin can be used along with NASIDs clinically to improve the anti-inflammatory effects of the NASIDs and to reduce their gastric side effects. The clinical trials for this combination definitely deserve to be tried.

AUTHORSHIP

Dr. DB and Dr. AC contributed to conception, revised the manuscript critically and approved it. Dr. PKP contributed to conception, prepared figures, drafted the manuscript and edited it. BB contributed in drafting of manuscript.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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