Stress-induced gastric ulcer and matrix metalloproteinases: Melatonin as rescuer

Snehasikta Swarnakar

ABSTRACT

Stress causes the generation of oxidative bursts that are accompanied by inflammation in different tissues, including gastric tissues. Over the period of two decades, my laboratory and others have reported the involvement of matrix metalloproteases (MMPs), mainly MMP-9 and -3 upregulation, in gastric ulcers developed by non-steroidal anti-inflammatory drugs (NSAIDs), Helicobacter pylori infection and stress. The excess gastric oxidative damage and MMP-3-dependent gastric inflammation played significant roles in stress-induced gastric mucosal injury. Moreover, the production of reactive oxygen species (ROS) is associated with mitochondrial dysfunction and apoptosis of gastric mucosal cells as assessed by depolarization of mitochondrial Δψm, caspase activity, and Bax protein expression in stress-induced gastric tissues of mice. Furthermore, stimulating redox-sensitive p38 and activating TNF-α and NF-κB caused MMP-3 and -9 upregulation at protein and activity levels in stress-induced ulcers. Experiments were performed to prevent stress ulcers by a small molecule named melatonin, a hormone having antioxidant and anti-inflammatory activity. Melatonin exerted MMP-3 inhibitory activity in an animal model of swim stress-induced gastric ulcer, which was supported by molecular dynamics simulations studies of the MMP-3-melatonin complex. Oxidative stress-induced damage was protected significantly better by melatonin-loaded nanocapsules (MNCs) as compared to free melatonin in cell culture systems. Additionally, restrained cold stress-induced gastric ulcers reduced MMP-2 activity that perturbs extracellular matrix rearrangement in gastric tissues. The drug omeprazole restored MMP-2 activity by binding with the MMP-2/TIMP-2 complex, thus maintaining the protease/anti-protease ratio while inhibiting gastric ulcers. Herein, the mechanism of stress-induced gastric ulcer, the involvement of MMPs thereon, and the role of melatonin in modulating MMP activity are discussed.

Keywords: Matrix metalloproteinases (MMPs), Zymogen, Gastric ulcer, Stress, Melatonin.

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INTRODUCTION

Mechanism of Gastric Ulcer Development

Stress causes the generation of oxidative bursts that are accompanied by inflammation in different tissues, including gastric tissues.[1] Over the period of two decades, my laboratory and others have reported the involvement of matrix metalloproteases (MMPs), mainly MMP-9 and -3 upregulation, in gastric ulcers developed by non-steroidal anti-inflammatory drugs (NSAIDs), Helicobacter pylori infection and stress.[2–5] The excess gastric oxidative damage and MMP-3-dependent gastric inflammation played significant roles in stress-induced gastric mucosal injury.[4] Moreover, the production of reactive oxygen species (ROS) is associated with mitochondrial dysfunction and apoptosis of gastric mucosal cells as assessed by depolarization of mitochondrial Δψm, caspase activity, and Bax protein expression in stress-induced gastric tissues of mice. Furthermore, stimulating redox-sensitive p38 and activating TNF-α and NF-κB caused MMP-3 and -9 upregulation at protein and activity levels in stress-induced ulcers. Experiments were performed to prevent stress ulcers by a small molecule named melatonin, a hormone having antioxidant and anti-inflammatory activity. Melatonin exerted MMP-3 inhibitory activity in an animal model of swim stress-induced gastric ulcer, which was supported by molecular dynamics simulations studies of the MMP-3-melatonin complex.[4] Oxidative stress-induced damage was protected significantly better by melatonin-loaded nanocapsules (MNCs) as compared to free melatonin in cell culture systems.[6] Additionally, restrained cold stress-induced gastric ulcers reduced MMP-2 activity that perturbs extracellular matrix rearrangement in gastric tissues. The drug omeprazole restored MMP-2 activity by binding with the MMP-2/TIMP-2 complex, thus maintaining the protease/anti-protease ratio while inhibiting gastric ulcers.[7] Herein, the mechanism of stress-induced gastric ulcer, the involvement of MMPs thereon, and the role of melatonin in modulating MMP activity are discussed.

In the modern era, most young people are stressed to cope with day-to-day demands. Stress is a major factor against cellular homeostasis for the development or aggravation of many diseases, including gastropathy. Several factors

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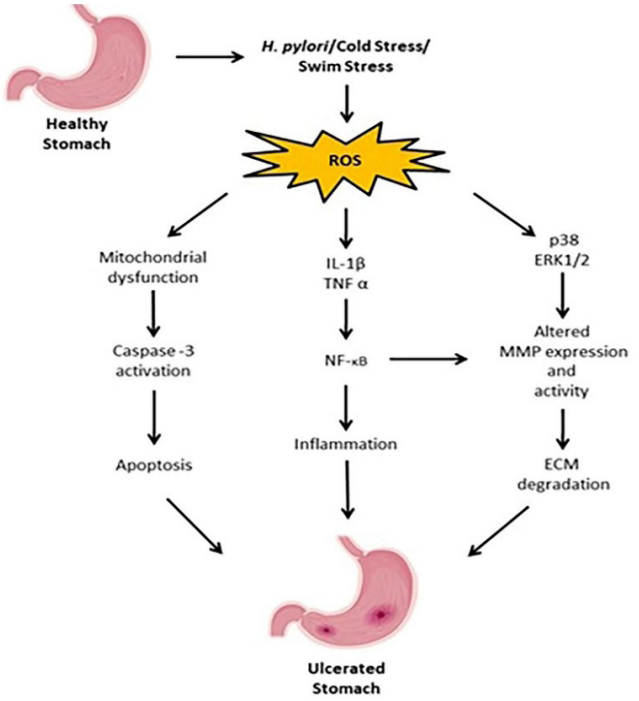

Figure 1: Mechanism of gastric ulcer development. Gastric ulcers can be developed by H. pylori infection, excess use of NSAIDs, alcohol, and stress (both physical and psychological). Generation of ROS is common for each pathway, leading to MMP modulation, ECM degradation, apoptosis, and inflammation. These different pathways are mentioned in this figure leading ultimately to gastric ulcer

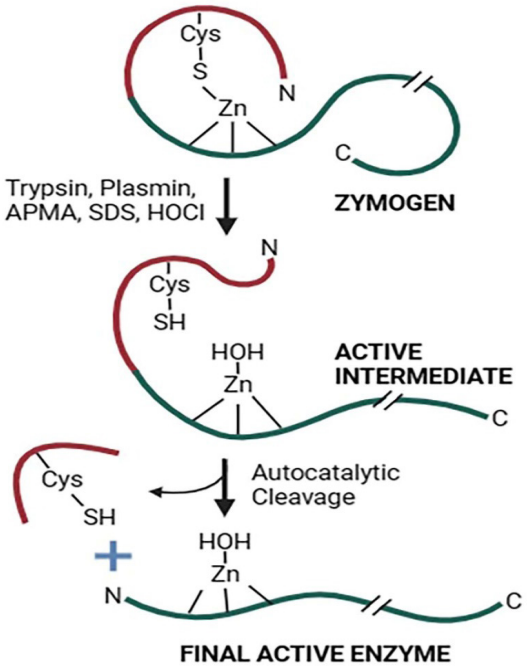

Figure 2: Matrix metalloproteinase activation scheme. MMPs secreted as zymogens maintain their latency through the interaction of cysteine in the pro-domain with zinc in the catalytic domain. Some chaotropic molecules make the zymogen into its active intermediate where interaction between the pro- and catalytic domains is absent. The final active enzyme is produced from the active intermediate by auto-catalytic cleavage when the pro-domain is cleaved

that cause gastric ulceration are stress, excess use of non-steroidal anti-inflammatory drugs (NSAIDs), and *Helicobacter pylori* infection. Gastric lesions may occur through several mechanisms, including inhibition of prostaglandin synthesis, depletion of glutathione, increase in IL-1 expression, increased lipid peroxidation, increase in the concentration of MMP-1, -9, and -3, activation of NFκB, and increase in apoptotic signaling pathways.

Nearly 30 million people all over the world take NSAIDs regularly for pain relief, although these drugs have several drawbacks. NSAIDs like ibuprofen, acetaminophen, or indomethacin cause gastric tissue damage by suppressing mucosal blood flow and prostaglandin synthesis, as well as reactive oxygen species (ROS) production. Both stress and NSAIDs play significant roles in developing gastric ulcers, encompassing acid secretion, ROS production, ECM remodeling, cell apoptosis, etc. ROS, such as superoxide (O^{2-}) and hydrogen peroxide (H_2O_2), can damage the cellular DNA, causing inflammation and tissue damage. Redox signaling also plays a central role in regulating cell function by altering the activity of metabolic enzymes and transcription factors, gene expression, and epigenetic alterations. MMPs are the enzymes attributed to the remodeling of ECM that are significantly associated with health and diseases. Among the different MMPs, mainly MMP-9 and MMP-3 are involved in inflammation by the breakdown of the basement membrane collagen. A considerable number of research works suggest that remodeling of ECM by gelatinases and collagenases is involved in gastric ulcers developed by NSAIDs/stress/*H. pylori* infection (Figure 1).

The role of MMPs in stress-induced ulceration is poorly known. Both MMP-dependent and independent pathways exist in gastropathy. Recent studies indicated the role of MMP-2 and MMP-3 in stress-induced gastric ulceration and the effect of melatonin thereon.

Overview of Matrix Metalloproteinases

Matrix metalloproteinases (MMPs) are a family of zinc-containing calcium-dependent extracellular matrix (ECM)

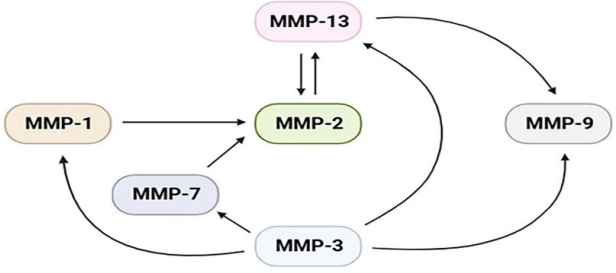

Figure 3: A brief overview of mutual interactions among different MMPs. MMP-3 can cleave pro-MMP-7, pro-MMP-9, or pro-MMP-13 to their active counterparts. Similarly, MMP-7 or MMP-13 can cleave pro-MMP-2 to active MMP-2 and so on as mentioned in the diagram

remodeling endopeptidases having 27 members. Based on their sub-cellular localization and substrate specificity for different ECM components, MMPs are divided into membrane-type matrix metalloproteases (MT-MMPs) (MMP-14, MMP-15, MMP-16, MMP-17, MMP-24, and MMP-25), collagenases (MMP-1, MMP-8, MMP-13, and MMP-18), gelatinases (MMP-2 and MMP-9), stromelysins (MMP-3, MMP-10, and MMP-11), and matrilysins (MMP-7 and MMP-26). MMPs are normally synthesized as inactive zymogens to prevent intracellular damage in healthy cells.[8] The only structural difference between the active and zymogenic forms of MMPs is the presence of the propeptide (or the prodomain). The sequencing data of MMPs suggest that a highly conserved cysteine residue is present in the propeptide domain of each MMP. This cysteine interacts with zinc in the catalytic domain to maintain enzyme latency. Physical agents like sodium dodecyl sulfate (SDS) can cause their tertiary structures to unfold, thereby exposing the zinc ion (Figure 2). Reagents that react with the sulfhydryl groups, like hypochlorous acid (HOCl) and aminophenyl mercuric acetate (APMA), can also inactivate this cysteine switch. On the other hand, proteolytic enzymes (including a few MMPs) interact with MMPs and induce cleavage of their pro-domain (Figure 3). In the second step, the activated metalloproteinases can autocatalytically cleave the active forms to eliminate the propeptide and impart permanent activity.[9]

MMPs and Gastric Inflammation

Gastric ulcers develop because of an imbalance between aggressive and protective factors in gastric tissues. Hydrochloric acid, pepsin, bile, ROS, and leukotriene are endogenous aggressive factors, and stress, *H. pylori* infection, NSAIDs, and alcohol are exogenous aggressive factors. Stress is one of the major culprits in generating

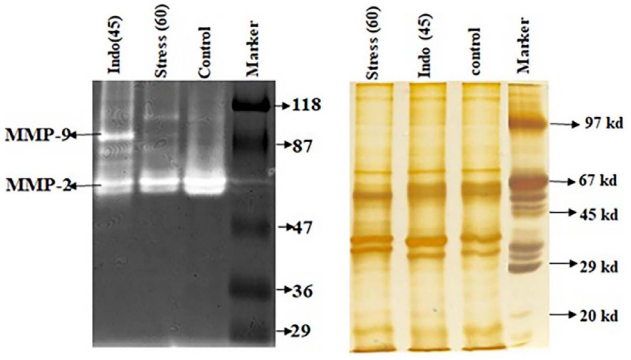

Figure 4: MMP profile of ulcerated tissue samples. Gastric ulcer was produced in Sprague-Dawley rats either by administration of indomethacin or restrain cold stress or none.[5, 7] After 3 hours of treatment, separate groups of animals were sacrificed, stomach tissues were isolated, and extracts in PBS buffer were made. An equal amount of proteins was run either in SDS-PAGE or gelatin-impregnated SDS-PAGE, followed by silver staining and Coomassie, respectively. Band patterns are different in indomethacin and stress-induced ulcerated tissues. White bands in gelatin gel signify that MMP-2 is reduced and MMP-9 is increased in ulcerated tissue extract

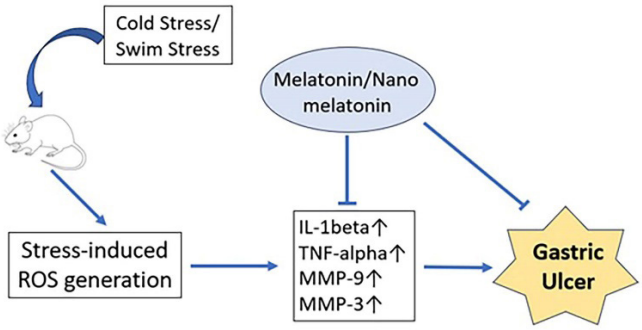

Figure 5: Schematic diagram of melatonin-mediated inhibition of stress-induced gastric ulceration. Either cold or swim stress causes ulceration in animals that produce ROS and damage the gastric tissue via increased expression of some inflammatory genes (namely IL-1β, TNF-α, MMP-9, MMP-3) and subsequently inhibition of those inflammatory genes by melatonin or MNCs prevent gastric ulcer

ROS, which causes mucosal damage in the stomach, thus developing gastric ulcers. ECM remodeling of gastric tissues occurs by the proteolytic enzymes, namely MMPs, a family of endopeptidases associated with numerous pathological processes. Hence, understanding the proteomic portrait of MMPs allows the underlying mechanism of gastric ulcers. More than two decades of research on MMPs establish their significant contribution to several cellular events in gastric ulcers. MMPs degrade ECM and regulate cell proliferation, differential migration, invasion, etc.

Hormones, growth factors, cytokines, and mutual interaction control the expression and activity of MMPs. Considerable evidence suggests the pivotal role of MMPs in gastric ulcers. However, the involvement of specific MMP in stress-induced gastric ulcers remains unexplored. Recent publications from my laboratory establish the upregulation of MMP-3 in swim stress-induced gastric ulcers in mice.[4] Melatonin (N- Acetyl- 5-methoxy tryptamine) pretreatment inhibited the stress ulcer by inhibiting MMP- 3 activity and expression. Interestingly, a reduced level of MMP-2 is observed in restraining cold stress-induced ulcers in rat models that are significantly reversed by the drug omeprazole.[7] The protease anti-protease balance is critical in stress ulcers which is properly restored by omeprazole. Moreover, increased activity and expression of MMP-9 are found in NSAID-induced, *H. pylori*-infected, or alcohol-induced gastric ulceration. As MMP-3 and -9 are inflammatory genes thus their upregulation helps to judge gastric inflammation. Because of the similarity in domain structure of all the MMPs becomes difficult to find specific inhibitors for catalytic domain per se. Hence, a specific inhibitor for MMP-3 or -9 may be a better choice as an anti-inflammatory drug (Figure 4).

Melatonin's Action In Ulcer Prevention

The hormone melatonin is secreted mostly from the pineal gland and the enterochromaffin cells of the gut. Melatonin possesses antioxidant, anti-inflammatory, and anti-aging effects and a role in sleep and circadian rhythmicity. The

regulatory effect of melatonin on MMP function is an emerging field of study. Our research shows that melatonin prevents stress-induced gastric ulcers by binding directly with MMP-9 and MMP-3. The half-maximal inhibitory concentration (IC_{50}) is ~100 μM. Since our system produces melatonin, it is non-toxic up to a certain concentration level. The binding of melatonin to MMP-9 occurs through several H-bonds, pi-pi interactions, water bridges, salt bridges, metal chelation, and dipolar bonds.

Melatonin has multi-faceted activity as we have seen in hyperglycemic conditions that melatonin can inhibit hyperglycemia-induced excessive AGS cell growth by stopping AGS cells in the G_0/G_1 phase by interacting with the ATP-binding region of CDK-2, therefore reducing its kinase activity.[10] Melatonin offers protection against various types of diseases through its anti-inflammatory activity.[11] It increases apoptosis in mice models of endometriosis, thus regressing the endometriotic lesion. Additionally, it plays a neuroprotective role via inhibition of intrinsic apoptotic pathways (Figure 5).

CONCLUSION AND FUTURE PERSPECTIVE

Gastric ulcers are one of the major health issues in developing countries because of the modern lifestyle, poor sanitation, and lack of nutritious food. According to the latest WHO data, about 5 to 6 million people are affected by gastric-related disorders each year. There has been a paradigm shift in recent years regarding the analysis of biochemical pathways for gastric ulcers independent of acid secretion. Recent reports suggest a strong connection between MMP activity and gastric ulceration. My laboratory provides the first experimental evidence that stress-induced gastric ulcer is associated with increased MMP-3 activity and decreased MMP-2 activity. The major challenge is to reverse the altered MMP activity by small molecule treatment and to mitigate gastric tissue damage. In this view, the novel approach to specifically inhibit inflammatory MMP gene transcription or translation by a drug is of the utmost need. The knowledge of MMP-3 and/ or -9 inhibition by melatonin in stress-induced ulceration paves the way for drug formulation having better potency and lesser side effects against gastric inflammation. Furthermore, exploring proteomic profiling of MMPs in ulcerated gastric tissues would be the future direction in deciphering the right biomarker for diagnosis and therapeutics.

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