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Original Contribution

Hydrogen peroxide-mediated downregulation of matrix metalloprotease-2 in indomethacin-induced acute gastric ulceration is blocked by melatonin and other antioxidants

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Abstract

Gastric mucosal damage is directly associated with extracellular matrix degradation in which matrix metalloproteinases (MMPs) play a crucial role. Remodeling of connective tissues and loss of tissue integrity due to the action of MMPs are reported in several inflammatory diseases, including gastric ulcer. Indomethacin-induced gastric ulceration involves the generation of reactive oxygen species (ROS) and a reduction in MMP-2 transcription and translation. Our aim was to identify the mechanism for suppression of MMP-2 activity by ROS during acute ulceration and further to examine the possible actions of antioxidants, especially melatonin, during healing. Melatonin (*N*-acetyl-5-methoxytryptamine) blocked hydroxyl radical and nitrite anion generation, protein oxidation, mucosal cell disruption, and MMP-2 downregulation. In addition, suppression of MMP-2 activity by H_2O_2 in a dose- and time-dependent manner in vitro is blocked by melatonin, omeprazole, and curcumin. We observed that melatonin and other antioxidants (e.g., curcumin and omeprazole) offered gastroprotection in vivo by upregulation of suppressed MMP-2 expression and activity at the level of secretion and synthesis. Moreover, antioxidants reversed the suppression of MMP-2 expression by upregulation of MT1-MMP and downregulation of TIMP-2. Hence, we hypothesize that antioxidants exerted protection against H_2O_2-mediated inactivation and downregulation of MMP-2 expression during onset of indomethacin-induced ulceration.

Keywords: Hydrogen peroxide; Reactive oxygen species; Matrix metalloproteinases; Melatonin; Nonsteroidal anti-inflammatory drugs; Extracellular matrix; Antioxidants; Free radicals

Inflammatory reactions induced by indomethacin are a significant source of reactive oxygen species (ROS). ROS, e.g., H_2O_2 and the superoxide radical ($O_2^{\bullet -}$), play a causative role in cancer, gastric injury, atherosclerosis, neurodegeneration, and arthritis. A significant source of ROS is the partial reduction of molecular oxygen by the autoxidation of flavoproteins, which subsequently generates $O_2^{\bullet -}$ and H_2O_2 [1,2]. A major pathway for metabolism of $O_2^{\bullet -}$ is its dismutation to H_2O_2 by superoxide dismutase (Eq. (1)). H_2O_2 is toxic to cells directly by attacking at the molecular level or indirectly by generating the highly reactive and toxic hydroxyl radical ($^{\bullet}OH$) [3] (Eq. (2)):

$$O_2^{\bullet -} + O_2^{\bullet -} + 2H^+ \rightarrow O_2 + H_2O_2, \quad (1)$$

$$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + {}^{\bullet}OH + HO^{\bullet}. \quad (2)$$

Inflammatory injury is associated with increased generation of ROS, among other mediators [4]. Among the ROS, H_2O_2 can remain for a long duration inside the cell and often performs the role of a second messenger for various physiological stimuli

Abbreviations: MMP, matrix metalloproteinase; ROS, reactive oxygen species; NSAID, nonsteroidal anti-inflammatory drug; ECM, extracellular matrix; bw, body weight; ip, intraperitoneal; $O_2^{\bullet -}$, superoxide radical; $^{\bullet}OH$, hydroxyl radical; TIMP, tissue inhibitor of metalloproteinase; RT-PCR, reverse transcriptase-PCR.

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such as angiotensin [5], inflammatory cytokines, growth factors [6], and transforming factors [7]. To control the destructive potential of ROS, cells have developed several defence mechanisms, including enzyme systems such as superoxide dismutases, catalase, glutathione peroxidase, and gastric peroxidases [8,9]. Indomethacin, a representative of the nonsteroidal anti-inflammatory drug (NSAID) family, causes gastric ulcers through various processes, including generation of ROS, inhibition of prostaglandin synthesis, infiltration of polymorphonuclear leukocytes, induction of apoptosis, and initiation of lipid peroxidation [10–13].

Gastric ulceration is often associated with the dysregulation of extracellular matrix (ECM) remodeling of gastric tissue. Matrix metalloproteinases (MMPs) that selectively degrade the components of the ECM, such as collagen IV, collagen V, gelatin, elastin, and fibronectin, play important roles in ECM remodeling [14]. MMPs are produced as latent enzymes and are activated by proteolytic cleavage of their prodomain. The prodomain contains a highly conserved cysteine residue that binds to the Zn atom of the catalytic domain, thus preserving latency [15]. Reactive oxygen and nitrogen species can regulate MMP activity in vitro and disrupt the balance of MMP activation and inactivation [16]. Dysregulation of this balance is implicated in the pathogenesis of a variety of diseases, including gastric ulcer. The traditional pathway for the inactivation of MMPs is interaction with endogenous tissue inhibitors of metalloproteinases (TIMPs) [17]. MMP-2 (72-kDa gelatinase) is unique among the MMPs because its expression is constitutive and its activation is associated with the balance between membrane type 1-MMP (MT1-MMP) and TIMP-2 [14,17]. Studies regarding the remodeling of ECM through modulation of MMPs in cell culture and an in vivo model suggest the involvement of MMP-1, -2, and -9 in indomethacin-induced gastric ulcers [18,19]. Our previous studies documented that MMP-9 plays an important role during gastric damage by indomethacin [20,21]. MMP-9 and -2 are differentially regulated in both acute and chronic ulceration in the rat [19,20]. MMP-9 is considered an inflammatory protein and increases with severity of disease and is regulated through NF-κB-mediated transcriptional control [19,22]. The MMP-2 gene has a different regulatory element in its promoter region compared to MMP-9 and therefore the two enzymes vary with respect to their biological function [23]. The expression and activity of both MMP-2 and MMP-9 occur during hypoxia-associated oxidative stress [24]. ROS generally modulate MMP activity either indirectly through redox-dependent regulation of MMP gene transcription or directly through modification of MMP structure [25,26]. However, the direct association of ROS with the regulation of MMPs during gastric ulceration is not well studied. Several in vivo and in vitro cell culture studies already hypothesize the activation of gelatinases through ROS or ROS generators when applied time and dose dependently [27,28]. It has been demonstrated that sublethal H_2O_2 exposure induces MMP-2 activity in human endothelial cells [29], whereas it downregulates MMP-2 activity in human retinal pigment epithelial cells [30]. It has also been reported that exogenous addition of H_2O_2 rapidly activates endothelial cell-associated MMPs [31].

Antioxidants generally act as radical scavengers, inhibiting lipid peroxidation and other free radical-mediated processes, and thereby protect gastric ulcer [20,21]. Melatonin, the major pineal secretory product, is a multifunctional hormone which influences circadian rhythms [32], induces seasonal reproduction [33,34], reduces jet lag, and acts as a sleep aid [35]. Melatonin is also found in the gastrointestinal tract and bile of rats and humans [36,37]. Also, melatonin is a well-known antioxidant and it protects cells and tissues from the deleterious effects of ROS, e.g., H_2O_2, hydroxyl radical, peroxyl radical, and hypochlorous acid [38–40]. Although its gastroprotective activity has been attributed to scavenging of various reactive oxygen and nitrogen species [40], little information is available regarding the effects of melatonin on MMP activity during ulcer prevention [21]. Our previous study documented that melatonin inhibits the activity of secreted and synthesized MMP-9 while blocking gastric ulceration. However, the effects of melatonin on the interplay between ROS and MMP-2 in gastric ulceration have not yet been determined. Several reports regarding H_2O_2-mediated transformation of signaling molecules document that antioxidants block MMP expression under many conditions. The expression of MMP-1 is effectively blocked by *N*-acetylcysteine, a precursor of glutathione [41,42], or by catalase [42]. In addition, antioxidants block the induction of MMP-9, -3, and -12 in H_2O_2-mediated diabetes mellitus and emphysema [43,44]. Moreover, glutathione inhibits H_2O_2-induced changes in MMP-2 and -9 activity in human fibroblasts and liver allografts, respectively [45,46].

Here we report that accumulation of ROS in indomethacin-induced acute ulceration leads to oxidative inactivation of MMP-2 as well as suppression of MMP-2 transcription and translation. The present study provides evidence that the activity of MMP-2 is suppressed in vitro by H_2O_2 in a dose- and time-dependent manner and melatonin blocks ROS generation and redox-dependent alteration of MMP-2 activity, thereby preventing disruption of gastric mucosal cells. It blocks H_2O_2-mediated suppression of MMP-2 expression and activity (latent and active) both in vivo and in vitro. Furthermore, melatonin as well as curcumin and omeprazole block suppression of MMP-2 activity at the levels of both secretion and synthesis, which are associated with upregulation of TIMP-2 and downregulation of MT1-MMP during gastroprotection. The study reveals for the first time a mechanistic basis of MMP-2 downregulation during indomethacin-induced acute ulceration and its protection by melatonin and other antioxidants.

Materials and methods

Materials

Gelatin from porcine skin, indomethacin, melatonin, curcumin, Triton X-100 (TX), protease inhibitors cocktail, gelatin fused with 4% beaded agarose, fast blue BB salt, benzenesulfinic acid, and 5-bromo-4-chloro-3-indolyl phosphate/nitroblue