Involvement of reactive oxygen species in gastric ulceration: Protection by melatonin

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Uncontrolled hydrochloric acid secretion and ulceration in the stomach due to various factors are serious global problems today. Although the mechanism of acid secretion from the parietal cell is now fairly known, the mechanism of gastric ulceration is still not clear today. Among various causes of gastric ulceration, lesions caused by stress, alcohol consumption, *Helicobacter pylori* infection and use of nonsteroidal antiinflammatory drugs have been shown to be mediated largely through the generation of reactive oxygen species especially hydroxyl radical (·OH). A number of excellent drugs have been proved useful in controlling hyperacidity and ulceration but their long term uses are not devoid of disturbing side-effects. Hence, the search is still on to find out a compound possessing antisecretory, antulcer and antioxidant properties which will serve as a powerful therapeutic agent to cure gastric hyperacidity and ulcer. This article describes the role of reactive oxygen species in gastric ulceration, drugs controlling them with their merits and demerits and, the role of melatonin, a pineal hormone in protecting the gastric lesions with a final commentary on how melatonin research with respect to gastric pathophysiology can be taken forward with a view to projecting this indole as a promising therapeutic agent to control gastric ulceration in humans.

Ulceration of the stomach mainly develops in the antral region due to lesions in the gastric mucosa. Epigastric pain is the common clinical feature and in severe cases blood appears in the vomitus. Since in majority of cases it is aggravated due to pepsin-hydrochloric acid, it is also termed as peptic ulcer. It is considered as one of the major human sufferings today affecting nearly 5% of the global population. Management of this painful disease, its prevention or cure is one of the challenging problems today.

Gastric ulcer develops when a balance between some aggressive and defensive (cytoprotective) factors is lost. The aggressive factors are either endogenous or exogenous in origin. The endogenous damaging factors are hydrochloric acid, pepsin, refluxed bile, leukotrienes and reactive oxygen species (ROS) such as $O_2^-$, $H_2O_2$ and $OH^\cdot$. The exogenous damaging factors mainly include alcohol, steroidal and nonsteroidal antiinflammatory drugs and drugs which stimulate gastric acid and pepsin secretion, stress and tension and *Helicobacter pylori*. The mucosal defense against these aggressive factors is contributed by mucous-bicarbonate barrier, surface active phospholipid, prostaglandin, mucosal blood flow, cell renewal and migration, antioxidants and antioxidant enzymes and some growth factors.

Reactive oxygen species, generated in the cells of aerobically respiring organisms due to many factors, have been implicated in pathogenesis of many human sufferings like Parkinson’s, Alzheimer, Huntington’s diseases and many other neurodegenerative conditions. The role of ROS as a causative factor in certain ischemic cardiovascular and pulmonary diseases, carcinogenesis and reproductive disorders have also been studied extensively. Participation of ROS in inducing cancer in many cases has stormed the scientific community in the last few years.

This article presents a comprehensive view on how ROS is involved in the generation of gastric ulceration. This is followed by a short account of the merits and demerits of a number of already marketed drugs for ameliorating the condition of gastric hyperacidity and ulceration. Finally, the role of pineal hormone, melatonin, in preventing gastric ulceration by scavenging the endogenous $OH^\cdot$ is described indicating the possible areas where extensive research works can be carried out to understand the critical importance of melatonin in gastroprotection with the ultimate goal of its therapeutic application.
Reactive oxygen species and gastric ulceration

The seemingly paradoxical consequences of the beneficial and harmful effects of oxygen (O2) have been shown for several decades. While more than 95% of the O2 taken in by the aerobic organisms is fully reduced to water (H2O) during the process of mitochondrial respiration, a small percentage (<5%) of the O2 consumed is converted to semireduced species, i.e., the superoxide anion radical (O2−), hydrogen peroxide (H2O2) and the hydroxyl radical (·OH)3. These species are collectively referred to as reactive oxygen species (ROS) which can be highly toxic, and their interactions often with cellular macromolecules bring about oxidative damage. The most toxic of the ROS is the ·OH which is often formed when O2− and H2O2 are exposed to the trace transition metals iron or copper via metal-catalyzed Haber-Weiss reaction:

Fe3+ + O2− → Fe2+ + O2

Fe2+ + H2O2 → Fe3+ + ·OH + OH−

The net result is therefore,

O2− + H2O2 → O2 + ·OH + OH−

The mechanism of ROS formation and how the cellular antioxidant systems defend against accumulating ROS have already been reviewed. Involvement of ROS in pathogenesis of gastric ulceration was first evident from the studies on ischemia-reoxygenation-induced gastric mucosal injury. A growing body of experimental and clinical evidence suggests that gastric mucosal damage by ethanof, nonsteroidal antiinflammatory drugs, and Helicobacter pylori is mediated through reactive oxygen species. Moreover ROS may play an important role in gastric ulceration induced by several kinds of stress. ROS also decreases the level of endogenous antioxidants such as GSH, α-tocopherol and ascorbate, and make the mucosa more prone to oxidative damage. The pathogenesis of gastric mucosal lesions by water immersion restraint stress and burn shock in rat is associated with increased lipid peroxidation. Systemic administration of glutathione or superoxide dismutase prevents water-immersion stress-induced ulceration. Cold-restraint stress alters the level of various damaging and cytoprotective factors of rat gastric mucosa to cause gastric ulceration.

Although the involvement of ROS in gastric lesions caused by various types of stress has been reported, detailed investigation on the role of ROS in cold-restraint stress-induced gastric ulceration has been limited. Moreover, very few studies have been undertaken to show the causal role of any specific oxygen-derived free radical in mediating gastric damage during stress. Stress-induced gastric ulceration which is associated with increased lipid peroxidation and depletion of endogenous GSH is due to increased formation of O2−, activation of SOD and inactivation of gastric peroxidase (GPO) — a condition suitable for generation of ·OH and formation of more reactive ·OH which causes antioxidant depletion and lipid peroxidation. The cytoprotective enzyme, prostaglandin synthetase (PGS), has also been shown to be inactivated leading to decreased defence against the aggressive factors. Yoshikawa et al. reported suppression of both lipid peroxidation and gastric mucosal injury induced by ischemia-reperfusion, after administration of SOD and catalase, indicating the role of ROS in the damage. Lipid peroxidation caused by ·OH is increased in gastric lesions induced by ethanol, indomethacin, ischemia-reperfusion, water immersion, and burn shock. Dimethyl sulfoxide (DMSO), a specific ·OH scavenger, reduces the gastric mucosal injury produced by ischemia, stress or ethanol, indicating a critical role of ·OH in the mucosal damage. It has been shown by direct measurement using DMSO that ·OH is really generated in the gastric mucosa under stress. Keeping the results of Das and Banerjee and some reported by others, the plausible role of ·OH in the generation of stress-ulcer as shown in the Scheme 1 has been proposed. Stress causes both sympathetic and parasympathetic stimulation of the stomach, which induces an increased motility and muscular contraction leading to vascular compression and mucosal ischemia. Sympathetic stimulation also causes direct arteriolar vasoconstriction and, thus greatly reduces the blood flow to the stomach leading to local hypoxia and near or actual "ischemia". The ischemic condition increases the leakage of O2 from mitochondrial electron transport chain and facilitates the availability of "redox-active" copper or iron. Increased O2− production leads to increased level of H2O2 (by the action of SOD), which, in conjunction with O2− generates ·OH via the metal catalyzed Haber-Weiss reaction. Hydroxyl radicals thus generated oxidizes important cellular constituents such as structural and functional proteins, membrane lipids, and depletes glutathione. Lipid peroxidation causes...
loss of membrane fluidity, impaired ion transport and membrane integrity, and finally loss of cellular functions. Stress also causes inactivation of prostaglandin synthetase leading to decreased biosynthesis of prostaglandin—the master molecule for gastrointestinal protection against all forms of insults to the mucosa. These factors in conjunction play a key role in stress-induced gastric ulceration.

The in vivo production of 'OH from O₂⁻ and H₂O₂ need the presence of redox-active transitional metals like iron or copper. The involvement of the metal ions in 'OH-mediated oxidative damage has been substantiated by earlier observation that both lipid peroxidation and mucosal injury were prevented by the administration of a non-toxic metal ion chelator, desferrioxamine (DFO). Interestingly, iron has been reported to play a critical role in ROS-mediated injury to gastric mucosa. Yajima et al. have shown that DFO and phenanthroline protect the cultured gastric mucosal cells from H₂O₂-mediated lipid peroxidation and cellular injury by chelating iron. However, the availability of metal ions for in vivo production of 'OH has been the subject of much debate. There are reports that during tissue ischemia, iron and copper are liberated from their respective storage proteins and participate in H₂O₂-mediated Fenton reaction. Increased gastric motility and vascular compression during stress, as mentioned earlier, leading to mucosal ischemia enhances the rate of O₂⁻ production, which is taken care of by the adaptive increase of mitochondrial SOD. In vivo activation of SOD by stress is completely prevented by α-amanitin, an established inhibitor of RNA polymerase, which controls the transcription of DNA to form mRNA. Thus stress causes an increased synthesis of SOD by elevating the level of its mRNA. Presumably, the increased level of O₂⁻ generated during ischemic stress stimulates SOD biosynthesis through gene transcription. However, the source of the increased O₂⁻ during the stress is not clear yet. The xanthine dehydrogenase-xanthine oxidase system, which is supposed to form O₂⁻ in ischemia-reperfusion tissue injury, is not the source in the stomach, as earlier studies failed to show any change in xanthine dehydrogenase level by stress. Moreover, allopurinol, a potent inhibitor of xanthine oxidase, fails to inhibit gastric mucosal injury induced by water-immersion stress. Mitochondria are known to be the alternative source of O₂⁻ within the cell. During low oxygen tension, the mitochondrial electron transport chain may remain in a reduced state and leak electrons to increase the flux of O₂⁻. This enhanced production of O₂⁻ may release iron or copper or both from their storage proteins and lead to the generation of 'OH via metal catalyzed Haber-Weiss reaction as described earlier. As these metal ions form complexes with the biological molecules, they serve as the redox-active centers for repeated production of 'OH, leading to the oxidative damage of the macromolecules at or near the metal binding site. 'OH generated in vivo not only peroxidizes membrane lipids but also oxidatively damages the critical cellular proteins, thus impairing the vital cellular functions. In the stomach of stressed animals, an increased level of the protein carbonyl content was detected which is an indicator of metal-catalyzed 'OH-dependent oxidation of proteins. Interestingly, gastric peroxidase (GPO), a major antioxidant enzyme of the gastric mucosa, was found to be inactivated during stress and antioxidants like glutathione (GSH), vitamin E and the metal ion chelator, DFO can not only prevent gastric ulceration and lipid peroxidation, but also protect the GPO. This suggests that metal ion-dependent generation of 'OH is involved in the inactivation of GPO and may, in part, contribute to the increased protein carbonyl content in the stomach under stress. Detailed studies using purified rat gastric peroxidase (GPO) have clearly demonstrated that oxidative damage of GPO has significant role in stress-
induced gastric ulceration. In stress ischemia, when generation of $O_2^-$ and $H_2O_2$ increases, the latter diffuses to Cu$^{2+}$ binding site and generates 'OH in a 'site-specific' manner causing oxidative damage of the enzyme. The presence of a copper binding motif in GPO seems critical, as it makes the enzyme prone to suicidal inactivation by its own substrate, $H_2O_2$, under adverse condition like stress, which causes accumulation of increased intracellular $H_2O_2$ through stimulation of SOD.

Ingestion of ethanol is the predisposing cause of acute hemorrhagic gastric erosions in human. Ethanol lowers the concentration of non-protein sulphhydrils specially glutathione, thereby exerting ulcerogenic effect by increasing ROS formation. Non-steroidal antiinflammatory drugs (NSAIDs) such as aspirin, indomethacin, ibuprofen etc., which are commonly used as pain killer in the treatment of rheumatoid arthritis and many other acute and chronic inflammatory conditions cause gastric mucosal damage. The best studied drug, aspirin, by inhibiting prostaglandin synthesis, interferes with protective mechanism such as mucus and bicarbonate secretion, surface epithelial hydrophobicity and mucosal blood flow. These changes permit backdiffusion of acid through the breached surfaces to destroy cells, capillaries and vein causing hemorrhagic ulcer. Enhancement of leukotriene synthesis by NSAIDs exhibits sufficient damaging effect. Aspirin also decreases mucosal ATP synthesis and cell turnover process. The changes brought about by NSAIDs, as described above, in totality can induce gastric damage through the generation of ROS and inhibiting cell proliferation. NSAIDs also inhibit gastric peroxidase and may increase mucosal $H_2O_2$ and 'OH level to cause oxidative mucosal damage. Antithyroid drugs specially mercaptoethylimidazole (MMI), which is used to treat hyperthyroidism, is a potent inducer of gastric acid secretion. The effect is partially mediated through increased liberation of histamine and possibly through increased level of $H_2O_2$ by irreversible inactivation of gastric peroxidase. MMI can induce acid secretion in vitro on isolated gastric mucosa or in gastric gland preparation where the effect can also be mimicked by the addition of micromolar concentration of $H_2O_2$. Since MMI augments both acid and luminal pepsin content, this drug is potentially dangerous to aggravate gastric ulcer.

**Drugs currently used to treat hyperacidity and gastric ulceration—their merits and demerits**

The secretion of hydrochloric acid (HCl) under normal physiological condition as and when required, does not cause any problem; but sustained hypersecretion is a grave condition which haunts its victims day and night. In some cases, it leads to a serious incapacitating pathological condition, gastric ulcer when the natural cytoprotection of the gastric mucosa is lost to combat the aggressive factors including ROS among others. Various drugs are available to control gastric hyperacidity and ulcers of the stomach. Among these, H$_2$-receptor blockers and proton pump (H$^+$-K$^+$-ATPase) inhibitors are most widely used today though their prolonged use are not without side effects.

Cimetidine, ranitidine, famotidine and nizatidine bind with the H$_2$ receptor of the parietal cell and block the histamine-stimulated acid secretion. These drugs, however, cannot totally block acid secretion due to other mechanisms involving cholinergic and gastrin receptors which are not directly related to H$_2$-receptor. Prolonged use of these drugs have been reported to affect the oxidative metabolism of different drugs, cause laxation, headache, dizziness, nausea, skin rashes and may exert weak antiandrogenic effect, loss of libido and impotence in male patients. Cimetidine and ranitidine have been reported to cause reversible mental confusion in sick elderly patients.

Omeprazole, a substituted benzimidazole, completely blocks acid secretion irreversibly in animal and human by interacting with the proton-pumping H$^+$-K$^+$-ATPase. The other substituted benzimidazoles have been reported to be less potent than omeprazole. However, omeprazole is not devoid of side effects. Reported side effects include headache, diarrhea and skin rash. A report from World Health Organization described the development of impotence and gynecomastia in some male subjects receiving omeprazole with other medications. However, long term use of omeprazole may cause block of acid secretion (achlorhydria) and hypergastrinemia. Moderate hyperplasia of gastric enterochromaffin-like cells has been observed in patients receiving omeprazole for a long time. In animals, omeprazole produces gastric carcinoma when continued for a long time, probably due to complete acid inhibition which stimulates gastrin production causing hyperplasia. However, no such change is observed in human cases. Omeprazole reduces the secretion, synthesis,
and gene expression of pepsinogen, which may create problem in protein digestion resulting in diarrhea. Omeprazole has been reported to interact with cytochrome P-450 system and may alter the metabolism of some drugs.

Protective role of melatonin in stress and drug-induced gastric ulceration

Despite the developments of the antisecretory-antulcer drugs mentioned above, scientists are still in search for a drug having antisecretory, antulcer and antioxidant properties which can be used to control the incidence of gastric ulceration in human with no or minimum toxicity. In this endeavour, an interesting observation was made suggesting that pineal hormone, melatonin [N-acetyl-5-methoxytryptamine] is capable of preventing stress and drug-induced gastric ulceration. The structure of melatonin is now known (Fig. 1). However, before describing its gastroprotective effects it will be worth mentioning the protective effects of melatonin in various other systems.

Being the principal secretory product of the pineal gland, melatonin influences circadian rhythmicity by acting on the suprachiasmatic nucleus. Additionally, melatonin influences various physiological activities such as neuroendocrine function, regulation of seasonal reproduction, possibly sexual maturaion, immunoregulation and thermoregulation.

Apart from the above functions, it is now widely claimed that melatonin possesses strong antioxidant activity by which it protects cells, tissues and organs from oxidative damage by reactive oxygen species, especially the "OH which attacks DNA, proteins and lipids and causes pathogenesis. Melatonin can scavenge the "OH generated in vitro by ultraviolet irradiation of H₂O₂. It can quench the peroxynitrite, peroxyl radical, hypochlorous acid and singlet oxygen, all of which cause cell damage. Melatonin also inhibits the production of nitric oxide (NO). Melatonin protects against age-related oxidative damage in the central nervous system, oxidative damage of the neuroblastoma cells by amyloid β protein which is characteristic of Alzheimer's diseases, 1-methyl-phenyl-1,2,4,6-tetrahydropyridine (MPTP) and iron-induced Parkinson-like neurodegenerative changes and free radical damage to outer hair cells in the organ of Corti. Melatonin also prevents oxidative damage of liver caused by ischemia-reperfusion as well as in lung and brain damage induced by hyperbaric oxygen. Melatonin was shown to reduce cardiac arrythmias and this action was attributed, at least in part, to its radical scavenging activity. Melatonin's inhibition of lipid peroxidation induced by δ-aminolevulinic acid which occurs in experimental porphyria as well as cataractogenesis induced by buthionine sulfoximine (an inhibitor of glutathione synthesis) in newborn rats, has also been documented. Moreover, endogenous melatonin has also been shown to play an important protective role against carrageenan-induced local inflammation. Furthermore, the protective effects of melatonin and related molecules against Cr (III)-induced carcinogenesis have been shown to be related to their direct "OH scavenging ability which thereby reduces the formation of the damaged DNA product, 8-hydroxydeoxy guanidine.

Apart from the pineal gland, melatonin is also present in high concentrations in the gastrointestinal tract, which may be a major source of melatonin. High levels of melatonin has been reported in bile. Melatonin binding sites have also been detected and characterized in stomach, jejunum, ileum and colon. Pigs with most severe ulcers exhibited significantly lower concentrations of melatonin in their stomach tissues and dietary supplementation of food with melatonin have been reported to reduce the incidence of gastric ulcers in pigs. Very recently, endogenous melatonin has been shown to be physiologically involved in the pre- and postprandial changes of intestinal motility at night. It is of interest as to why the gastrointestinal tract and the bile contain such high concentrations of melatonin.

Interest has been focussed on the role of melatonin as an antioxidant and its protection of gastric mucosa against oxidative damage caused by ROS in different experimental models of ulcer. Melatonin can protect against ethanol-induced gastric damage by...
preventing inflammation caused by accumulation of polymorphonuclear neutrophils, by ameliorating the decrease in GSH level and by decreasing the activity of GSH reductase. The latter two systems are intimately involved in the protection of the cells against free radical-induced damage. Melatonin also prevents gastric mucosal lesions caused by water-immersion restraint stress, ischemia and aspirin, and this is accompanied by an elevation in gastric level of prostaglandin, an important gastroprotective factor, and a marked fall in blood free radicals as measured by chemiluminescence. Melatonin also prevents ischemia/reperfusion-induced gastric lesions by reducing lipid peroxidation, limiting the decrease in GSH peroxidase activity and by causing a reduction in neutrophil accumulation. These works clearly demonstrate beyond doubt that melatonin has strong gastroprotective properties. Detailed studies on the antiulcer effect of melatonin have revealed several interesting data. Table 2 shows the effect of administration of melatonin in rats on cold-restraint stress-induced gastric lesions as indicated by the ulcer index. Melatonin dose-dependently protected the mucosa from stress-induced ulceration, causing 87% inhibition of the ulcer index when given at a dose of 60 mg/kg bw (ip). The effect of melatonin was compared with known antiulcer compounds such as ranitidine and omeprazole and the potencies (ED50, i.e. the dose required for 50% inhibition) of these compounds in preventing stress-ulcers are shown in Table 3. The results indicate that melatonin is almost three times more potent than ranitidine although it is less potent than omeprazole.

As stress ulcer is associated with oxidative membrane damage caused by OH-induced lipid peroxidation, the effect of melatonin on lipid peroxide level was measured.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of animals</th>
<th>Ulcer index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-stressed control</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Restraint-cold stress</td>
<td>15</td>
<td>59.3 ± 1.9</td>
</tr>
<tr>
<td>Restraint-cold stress + melatonin (20mg/kg)</td>
<td>9</td>
<td>17.6 ± 2.4*</td>
</tr>
<tr>
<td>Restraint-cold stress + melatonin (40mg/kg)</td>
<td>14</td>
<td>12.9 ± 3.1**</td>
</tr>
<tr>
<td>Restraint-cold stress + melatonin (60mg/kg)</td>
<td>15</td>
<td>6.7 ± 1.9**</td>
</tr>
</tbody>
</table>

*P values: *<0.02; **<0.001

Table 2 - Effect of melatonin on restraint-cold stress-induced gastric ulceration

[Values are mean ± SE]

<table>
<thead>
<tr>
<th>Experiments carried out</th>
<th>Parameters measured</th>
<th>Effect</th>
<th>Effect after melatonin treatment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ischemia-reperfusion induced gastric lesion</td>
<td>Lesion area</td>
<td>Increased</td>
<td>Decreased</td>
<td>95</td>
</tr>
<tr>
<td>Ischemia-reperfusion induced gastric lesion</td>
<td>Basal acid secretion</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Ischemia-reperfusion induced gastric lesion</td>
<td>Blood free radicals</td>
<td>Increased</td>
<td>Decreased</td>
<td></td>
</tr>
<tr>
<td>Ischemia-reperfusion induced gastric lesion</td>
<td>Gastric mucosal lipid peroxidation</td>
<td>Increased</td>
<td>Decreased</td>
<td>97</td>
</tr>
<tr>
<td>Ischemia-reperfusion induced gastric lesion</td>
<td>Neutrophil infiltrated myeloperoxidase activity in gastric mucosa</td>
<td>Increased</td>
<td>Decreased</td>
<td></td>
</tr>
<tr>
<td>Ischemia-reperfusion induced gastric lesion</td>
<td>Glutathione peroxidase activity of gastric mucosa</td>
<td>Decreased</td>
<td>Increased</td>
<td></td>
</tr>
<tr>
<td>Ischemia-reperfusion induced gastric lesion</td>
<td>Acid output</td>
<td>—</td>
<td>Decreased</td>
<td>94</td>
</tr>
<tr>
<td>Ischemia-reperfusion induced gastric lesion</td>
<td>Basal pepsin</td>
<td>—</td>
<td>Decreased</td>
<td>93</td>
</tr>
<tr>
<td>Ischemia-reperfusion induced gastric lesion</td>
<td>Basal gastrin</td>
<td>—</td>
<td>Increased</td>
<td></td>
</tr>
<tr>
<td>Ischemia-reperfusion induced gastric lesion</td>
<td>Gastric epithelium</td>
<td>Damaged</td>
<td>Prevented</td>
<td></td>
</tr>
<tr>
<td>Water-restraint stress induced damage</td>
<td>Gastric lesion</td>
<td>Increased</td>
<td>Decreased</td>
<td>94</td>
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<tr>
<td>Water-restraint stress induced damage</td>
<td>DNA synthesis</td>
<td>Decreased</td>
<td>Increased</td>
<td>93</td>
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<tr>
<td>Water-restraint stress induced damage</td>
<td>Gastric blood flow</td>
<td>Reduced</td>
<td>Increased</td>
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<td>Water-restraint stress induced damage</td>
<td>PGE2 levels</td>
<td>Decreased</td>
<td>Increased</td>
<td></td>
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<tr>
<td>Water-restraint stress induced damage</td>
<td>Blood free radicals</td>
<td>Increased</td>
<td>Decreased</td>
<td></td>
</tr>
<tr>
<td>Ethanol-induced Gastric damage</td>
<td>PMN infiltration</td>
<td>Increased</td>
<td>Decreased</td>
<td>92</td>
</tr>
<tr>
<td>Ethanol-induced Gastric damage</td>
<td>Glutathione level</td>
<td>Decreased</td>
<td>Restored</td>
<td></td>
</tr>
<tr>
<td>Ethanol-induced Gastric damage</td>
<td>Glutathione reductase activity</td>
<td>Decreased</td>
<td>Restored</td>
<td></td>
</tr>
</tbody>
</table>
ured. The protein carbonyl content and reduced glutathione level were also measured, as these parameters were found to change in oxidative stress. These parameters were studied in order to demonstrate the ability of this indole to prevent oxidative stress-induced changes. The results as shown in Table 4 indicate that stress-induced ulcers are associated with an increase in lipid peroxidation products, protein carbonyl content and a decrease in the level of reduced glutathione. Melatonin, which caused an 87% reduction in the stress-ulcer index, also brought down the lipid peroxidation, prevented oxidation of protein as well as helped keep glutathione content of the gastric mucosa to normal level. All these observations naturally establish the antioxidative function of melatonin in preventing the stress-induced gastric ulceration in rats.

As melatonin is now regarded both as a direct and an indirect antioxidant, the question that naturally arises is whether melatonin's protective role against stress-induced gastric ulceration is a consequence of the indole's direct effect i.e., whether it affords protection by quenching the reactive oxygen species like \( \cdot \text{OH} \) or \( \text{H}_2\text{O}_2 \) or by an indirect mechanism such as altering the activities of the enzymes responsible for the metabolism of reactive oxygen species thereby reducing the possibility of accumulation of ROS in the face of oxidative-stress. Studies indicate that melatonin prevents stress-induced gastric ulcer in vivo by directly scavenging the \( \cdot \text{OH} \) (Table 5). The imposed stress caused an almost 6-fold increase in the tissue level of \( \cdot \text{OH} \), while melatonin administration led to a significant reduction. The result suggests that melatonin inhibits the endogenous level of \( \cdot \text{OH} \) during the restraint-cold stress and protects the stomach from ulceration. This observation prompted us to find out whether melatonin directly quenches the \( \cdot \text{OH} \) or it indirectly inhibits the formation of \( \cdot \text{OH} \) by altering some antioxidant enzyme level. The \( \cdot \text{OH} \) scavenging ability of melatonin was studied in vitro where \( \cdot \text{OH} \) was generated with \( \text{Cu}^{2+}/\text{ascorbate} \), an established \( \cdot \text{OH} \) generating system. Table 6 indicates that melatonin can directly scavenge \( \cdot \text{OH} \) in a concentration dependent manner exhibiting 81% scavenging activity at a concentration of 100 \( \mu \text{M} \) with an \( EC_{50} \) value of 40 \( \mu \text{M} \).

Since \( \cdot \text{OH} \) are generated by the metal catalyzed Haber-Weiss reaction involving \( \text{O}_3 \) and \( \text{H}_2\text{O}_2 \), the possibility exists that melatonin could reduce \( \cdot \text{OH} \) formation by scavenging \( \text{O}_3 \) and \( \text{H}_2\text{O}_2 \) or by chelating the redox active metal ions. Experiments have shown that...
melatonin neither scavenges $O_2^*$ nor $H_2O_2$\textsuperscript{57}. Further, melatonin also does not possess any metal ion chelating activity\textsuperscript{57}. Thus melatonin protects the stomach from stress-induced ulceration by scavenging the endogenous $^*$OH. Melatonin was proposed to scavenge $^*$OH by donating an electron to the latter resulting in the generation of indolyl cation radical\textsuperscript{98}. This radical product was proposed to interact with $O_2^*$ to produce N$^1$-acetyl-N$^2$-formyl-5'-methoxykynuramine. Although scavenging of $^*$OH via electron donation has not been totally ruled out, recent evidence indicates the formation of cyclic 3-hydroxymelatonin as a stable product which is excreted in the urine\textsuperscript{99,100}. This provides direct evidence that melatonin under physiological conditions, acts as an antioxidant to detoxify the most cytotoxic endogenous $^*$OH. In \textit{in vivo} studies further indicate that in the protection against stress ulcer, melatonin is superior to GSH and essentially equipotent to vitamin E, the established cellular antioxidants involved in the antioxidant defence\textsuperscript{57}. The results indicate that melatonin may be a more versatile radical scavenging antioxidant when compared with the classical antioxidants\textsuperscript{57}.

Indomethacin (IMN), an NSAID, is known to cause gastric lesions by decreasing prostaglandin level through inhibition of prostaglandin synthesis. The experiments revealed that melatonin dose-dependently prevents IMN-induced gastric ulceration\textsuperscript{57} indicating that it may be involved in maintaining endogenous prostaglandin level which offers gastrogtection by inhibiting acid secretion, increasing mucus and bicarbonate secretion, elevating blood flow and by other gastroprotective mechanisms\textsuperscript{96}. Similar effects of melatonin on IMN-induced gastric damage have also been reported\textsuperscript{101}.

**IMN** is an established inhibitor of the cyclooxygenase, a component of prostaglandin synthetase, involved in prostaglandin biosynthesis. How melatonin prevents IMN action is not known yet. Further studies are required to clarify this.

**Future perspectives**

Modern state-of-the-art researches on gastropathophysiology have documented some exciting observations. The maintenance of gastric mucosal integrity depends on the interplay between epithelial cell proliferation and apoptosis (i.e., programmed cell death). The Bcl-2 family of proteins plays a central role in the regulation of apoptotic cell death by suppressing the apoptosis, while some others such as Bax proteins promote this process\textsuperscript{102}. Therefore, it is conceivable that stress-induced gastric ulcerations are accompanied by the fall in gastric mucosal cell proliferation. Studies by Konturek \textit{et al.}\textsuperscript{102} have yielded information about the influence of the stress on the apoptosis in gastric mucosa. Through their nicely designed studies, they concluded that healing of gastric lesions involves an increase in gastric mucosal blood flow and mucosal cell proliferation, and, the enhancement in gastric epithelial apoptosis accompanies the mucosal damage induced by stress and this appears to be triggered by the shift from cell death effector Bax to the cell death repressor Bcl-2 protein\textsuperscript{102}.

Gastric mucosal apoptosis is characterized by a series of distinct biochemical and morphological changes which involve activation of caspase proteases cascade that remains under the regulatory control of nitric oxide (NO). The results of Slomiany \textit{et al.}\textsuperscript{103} implicated caspase-3 in the process of indomethacin-induced gastric epithelial cell apoptosis, and point towards participation of nitric oxide synthetase-2 (NOS-2) in the amplification of the cell death signalling cascade\textsuperscript{103}. Additionally, indomethacin, sodium diclofenac, flurbiprofen, zaltoprofen, etodolac, but not mofoezolac, have been found to enhance apoptotic DNA fragmentation and mRNA expression for cyclooxygenase-2 in AGS cells, a cell line derived from human gastric epithelium. Further, lucigenin chemiluminescence showed that intracellular production of ROS increased in cells treated with indomethacin. These observations thus indicate a crucial association between the generation of ROS and NSAID-induced apoptosis in gastric epithelial cells\textsuperscript{104}. Moreover, it has been envisaged that during ulcer healing, an array of factors compel mucosal cells to proliferate, differentiate, and migrate to the site of injury. The recognition of triggering cues requires close interaction between the regulatory proteins integrating the growth factor and cytokine-mediated signals that propel cells through the cycle events, or to signal apoptosis. Investigations by Slomiany \textit{et. al.}\textsuperscript{105} provide strong indications that the initial phase of ul-

<table>
<thead>
<tr>
<th>System</th>
<th>nmol $^*$OH generated/ml reaction mixture</th>
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<tbody>
<tr>
<td>Control</td>
<td>544 ± 20</td>
</tr>
<tr>
<td>+Melatonin</td>
<td></td>
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<tr>
<td>25µM</td>
<td>486 ± 42</td>
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<tr>
<td>50µM</td>
<td>196 ± 23</td>
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<tr>
<td>100µM</td>
<td>92 ± 6.4</td>
</tr>
<tr>
<td>250µM</td>
<td>84 ± 11</td>
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cer healing involves the inhibition of apoptotic caspase activities by signalling events initiated by bFGF-receptor activation and propagated by the regulatory kinases that propel the cell cycle progression. Their findings strongly point towards participation of cNOS in the suppression of proapoptotic activities in gastric mucosa.

Therefore, conceivably, when oxidative stress ensues in the gastric mucosa, a group of cells die when their antioxidant defense system is overwhelmed by the accumulating ROS, and another group partially damaged also die, in anticipation of further onslaught, through the process of apoptosis which may be triggered by the ROS themselves acting as a second messenger. However, possibility of cells dying of necrosis always remain viable under such conditions. Whatever be the death pathway, the final outcome is the ulceration of the gastric mucosa following oxidative damage due to ROS.

The answer to the question of how melatonin affords gastroprotection through the scavenging of ROS seems available by this time. However, the answer to the question of how melatonin provides protection against oxidative damage due to stress through its indirect antioxidant effects in other systems is gradually coming up although lacunae exist as regards such mechanisms by which this indole could provide gastroprotection. Kotler et al. examined the influence of melatonin on gene expression for antioxidant enzymes in rat brain cortex. Their results clearly demonstrate that exogenously administered melatonin increases the levels of mRNA for glutathione peroxidase, copper-zinc SOD, and Mn-SOD in this tissue. These stimulatory effects are observed after both acute and chronic treatment with this hormone, producing in the latter case a more marked increase thereby indicating that melatonin exerts an important role in providing indirect protection against free radical injury by stimulating gene expression.

The role of melatonin in the regulation of apoptosis was studied by Sainz and coworkers. They investigated the role of melatonin in the cell death of thymus, a well known model for the study of apoptosis. Both in vivo and in vitro experiments were carried out by them. Morphometrical studies in semithin sections of thymus and analysis of DNA fragmentation by gel electrophoresis showed that physiological apoptosis occurring in thymus of 65 days old rats, was prevented by the daily administration of melatonin beginning when the rats were 25 days old. However, they have not ruled out the possibilities of direct interaction of melatonin with glucocorticoid receptors in the thymus, induction of interleukin-4 release, direct genomic action modulating the expression of apoptosis inhibiting genes, an effect on nitric oxide synthase and finally the antioxidant action of melatonin. Later, these workers have, however, shown that melatonin has a major role in downregulating the glucocorticoid receptor and this downregulation is the likely mediator of its thymocyte protection against dexamethasone-induced cell death. This effect of melatonin, given the antioxidant properties of glucocorticoids, adds another mechanism to explain its antioxidant effects.

The future work relating to the role of melatonin on the gastroprotection may address the following questions.

1. Does melatonin influence the antioxidant enzymes of the gastric mucosa directly or through enhancing their gene expression?
2. Does melatonin also exhibit the antiapoptotic effect on gastric mucosal cells? It will be highly interesting to explore how and to what extent melatonin influences the balance of expression of Bcl-2 and Bax proteins of gastric mucosal cells.
3. Does melatonin enhance angiogenesis and proliferation of surface epithelial cells of the stomach? Because such actions will trigger healing of ulcer.
4. Does melatonin influence the expression of transcription factors (like NF-KB) as these under certain conditions may induce mucosal apoptosis.
5. Attempts may be made to understand how and why melatonin gets concentrated in the stomach. What is the significance of gastric synthesis of melatonin because it is the stomach where the highest concentrations of melatonin are found among other portions of the gastrointestinal tract. Does this melatonin have any protective effect on the stomach against oxidative stress under physiological condition?
6. What are the functions of the melatonin receptors present in the stomach? Does binding of melatonin with its receptor in the stomach triggers any signal transduction pathway, if so, what message is transduced and what are its implications?
7. It will be interesting to investigate whether melatonin, after reaching the nucleus induces the expression of some hither-to-unknown melatonin responsive element(s) that perhaps may bear gastroprotective properties.
8. It will be worth investigating the influence of me-
latonin on prostaglandin biosynthesis for its gastroprotective effect.

9 Absence of any reported toxicity of melatonin in different animal models as well as in human makes this indole more interesting and subject of intense research. Under this situation it is reasonable to argue whether melatonin can increase the efficacy of the already marketed antiulcer drugs (ranitidine, omeprazol) by reducing their dose requirement or toxicity or bioavailability or all.

10 What will be an optimum (safe) dose that will not only protect patients from ulceration but also prevent damage of other organs from oxidative stress as general antioxidant?—a question easy to imagine but difficult to resolve.

The advantage of melatonin as an antioxidant lies in the fact that it is amphiphilic in nature109-111, thus, it can reach every compartment of a cell to scavenge the 'OH generated in the face of any oxidative stress. However, its 'OH scavenging ability, even at physiological concentrations, and absence of any demonstrated (short- or long-term) toxicity at pharmacological concentrations 112,113 may predictably make this small indole an important therapeutic molecule in future. This contention is strengthened further by the fact that it has also been tested, in humans, most frequently for its ability to reduce the symptoms of jet lag114, adjust 24 hour rhythmicity115 and as a sleep aid116. Presently, in U.S.A. and in some of the western countries, melatonin is being sold as an over-the-counter drug as an antioxidant as well as a sleeping aid (personal communication with Prof. Russel J. Reiter). Protection against stress-induced ulcers in our model and the protection afforded against the ischemia-reperfusion, aspirin and water-restraint stress-induced gastric ulcers by melatonin 93-95, 101,117 could be instrumental in significantly reducing gastric ulcers in humans and it may have utility in other areas as well66,73.

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