A Novel Antioxidant and Antiapoptotic Role of Omeprazole to Block Gastric Ulcer through Scavenging of Hydroxyl Radical*

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The mechanism of the antiulcer effect of omeprazole was studied placing emphasis on its role to block oxidative damage and apoptosis during ulceration. Dose-response studies on gastroprotection in stress and indomethacin-induced ulcer and inhibition of pylorus ligation-induced acid secretion indicate that omeprazole significantly blocks gastric lesions at lower dose (2.5 mg/kg) without inhibiting acid secretion, suggesting an independent mechanism for its antiulcer effect. Time course studies on gastroprotection and acid reduction also indicate that omeprazole almost completely blocks lesions at 1 h when acid inhibition is partial. The severity of lesions correlates well with the increased level of endogenous hydroxyl radical (•OH), which when scavenged by dimethyl sulfoxide causes around 90% reduction of the lesion, indicating that •OH plays a major role in gastric damage. Omeprazole blocks stress-induced increased generation of •OH and associated lipid peroxidation and protein oxidation, indicating that its antioxidant role plays a major part in preventing oxidative damage. Omeprazole also prevents stress-induced DNA fragmentation, suggesting its antiapoptotic role to block cell death during ulceration. The oxidative damage of DNA by •OH generated in vitro is also protected by omeprazole or its analogue, lansoprazole. Lansoprazole when incubated in a •OH-generating system scavenges •OH to produce four oxidation products of which the major one in mass spectroscopy shows a molecular ion peak at m/z 439, which is 16 mass units higher than that of lansoprazole (m/z 389). The product shows no additional aromatic proton signal for aromatic hydroxylation in 1H NMR. The product absorbing at 278 nm shows no alkaline shift for phenols, thereby excluding the formation of hydroxylansoprazole. The product is assigned to lansoprazole sulfone formed by the addition of one oxygen atom at the sulfur center following attack by the •OH. Thus, omeprazole plays a significant role in gastroprotection by acting as a potent antioxidant and antiapoptotic molecule.

Proton pump inhibitors (1) such as omeprazole, lansoprazole, pantoprazole, and rabeprazole are extensively used for therapeutical control of acid-related disorders including gastroesophageal reflux disease and Zollinger-Ellison syndrome and for peptic-ulcer disease caused by stress (stress-related erosive syndrome), nonsteroidal antiinflammatory drugs, and Helicobacter pylori infection (2–5). These compounds share a common structural motif contributed by a substituted pyridylmethylsulfinyl benzimidazole (Fig. 1). Inhibition of gastric acid secretion by these compounds is considered to be an important step to control the disorders (6). Proton pump inhibitors inhibit acid secretion by irreversibly interacting with the H+-K+-ATPase, the terminal proton pump of the parietal cell (7, 8). In the acid space of the secreting parietal cell or in the vicinity of the enzyme, these compounds are converted to thiophilic sulfenamide or sulfenic acid, which reacts mainly with the Cys-813 residue in the catalytic subunit of the H+-K+-ATPase, which is critical for enzyme inactivation (5). Although omeprazole, the primary member of the proton pump inhibitors, has been extensively used to control these disorders (2), lansoprazole, the second member of the substituted benzimidazole containing a trifluoroethoxy group, has also been used more recently (4).

The role of acid in gastroduodenal pathogenesis has been extensively studied. Although gastric ulcer patients show normal or reduced level of acid secretion, duodenal ulcer patients usually secrete more acid (9, 10). In fact, “no acid, no ulcer” is the dictum for duodenal ulcer. Because 30% of patients having duodenal ulcer and very few patients with gastric ulcer are hyperchlorhydric (9), clearly factors other than acid are involved in the pathogenesis of gastroduodenal ulcer. Although the secreted acid itself is not sufficient for ulcer formation, its corrosive property and increased peptic activity is sufficient to aggravate the ulcer. Even the normal rate of acid secretion may cause ulceration in the breached mucosa when some gastroprotective factors are lost. Hence, acid suppression by omeprazole is a common practice to control gastroduodenal lesions (2, 5). Suppression of intragastric acid also helps in the healing of ulcer (11). In animals, the role of acid in gastric lesions has been studied using some animal models such as stress or nonsteroidal antiinflammatory drug-induced gastric ulcer. Stress itself inhibits gastric acid secretion through a central nervous reflex mechanism (12). Restraint cold stress or restraint water immersion stress induces gastric lesions, which are associated with a decreased or normal level of acid secretion (13, 14). Because restraint or water immersion stress significantly decreases acid secretion induced by pylorus ligation (14), acid plays a minor role in stress ulcer. Administration of antacids to neutralize secreted acid does not protect stress ulcer (15), suggesting that factors other than acid are involved in ulcer formation. However, in indomethacin-induced gastric damage, acidity may be increased because of decreased biosynthesis of prostaglandin (16, 17). Because acidity as high as 0.6 M HCl can...
controlling oxidative damage and apoptotic cell death of the gastric mucosa during ulceration. The role of acid suppression effect of omeprazole on gastroprotection against some necrotizing agents (ethanol, acidified aspirin, hypertonic saline, 0.6 m HCl) has been studied earlier (18, 53), where evidence has been provided to show that acid inhibition plays no significant role on the gastroprotective effect of omeprazole. Moreover, omeprazole neither stimulates prostaglandin biosynthesis nor increases bicarbonate secretion to offer gastroprotection (18, 53). Thus, omeprazole exerts its antiulcer activity through some other mechanism that has not been explored yet. Using animal models of stress and indomethacin-induced gastric lesions and pylorus ligation-induced acid secretion, evidence has been presented in this paper to show that the gastroprotective effect of omeprazole is not mediated through its acid inhibitory effect. Further evidence has been presented to show that endogenous ‘OH plays one of the major roles in gastric lesions and that omeprazole acts as a potent antioxidant to scavenge the endogenous ‘OH, thereby preventing the oxidative damage by increased lipid peroxidation and protein oxidation. Moreover, it offers an antiapoptotic effect by blocking DNA fragmentation during ulceration. Evidence has also been presented to show that omeprazole or lanosoprazole blocks ‘OH-induced oxidative damage of DNA by scavenging ‘OH in vitro. Analysis of the major oxidation product of lanosoprazole indicates that this antioxidant activity is due to scavenging of ‘OH to form an oxygenated product that is assigned to lanosoprazole sulfone. The studies thus provide new insights on the mechanism of the antiulcer effect of proton pump inhibitors.

MATERIALS AND METHODS

**Drugs and Chemicals—** Omeprazole was a kind gift from Dr. W. Beil (Medizinische Hochschule, Hannover, Germany). Lansoprazole, melatonin, α-phenyl N-tetrahydroxynitroso- (PBN), thioribfuric acid, ethidium bromide, ascorbic acid, 2,4-dinitrophenylhydrazine, collagenase type 1A, Pronase E, proteinase K, RNase, catalase, guanidine HCl, Fast Blue BB salt, tetraethoxypropane, benzenesulfinic acid, and 5,5-dimethyl-1-pyrroline N-oxide (DMPO) were purchased from Sigma. Desferrioxamine was obtained from Ciba Geigy Ltd. Vitamin E (α-tocopheryl acetate), MeSO, and TLC plates coated with silica gel 60 F254 were procured from Merck.

**Animals Used—** Sprague-Dawley rats (200–250 g) of both control and experimental groups kept separately in controlled condition were fasted for 24 h with water ad libitum. The control group received the vehicle only while the experimental group received omeprazole intraperitoneally 30 min prior to restraint cold stress or indomethacin administration for gastric ulceration or pylorus ligation for acid secretion. Animal experiments (n = 8–30) were carried out following the guidelines of the animal ethics committee of the institute. Human gastric mucosal biopsy specimens were obtained from the Cancer Centre Welfare Home and Research Institute (Kolkata, India) following approval by the human ethics committee of the institute.

**Restraint Cold Stress-induced Gastric Ulceration—** The rats were immobilized under light ether anesthesia and subjected to cold (4 ± 1°C) stress for 3.5 h (13). The severity of mucosal lesions was scored as ulcer index as follows: 0 = no pathology; 1 = a small ulcer (1–2 mm); 2 = a medium ulcer (3–4 mm); 4 = a large ulcer (5–6 mm); and 8 = larger ulcer (>6 mm). The sum of the total scores divided by the number of animals was expressed as the mean ulcer index (13). Luminal acid content was determined by titration with 1 m NaOH using an autoburette pH stat system from Radiometer (Copenhagen, Denmark) (13).

**Indomethacin-induced Gastric Ulceration—** The rats were orally fed with indomethacin at 48 mg/kg of body weight. After 4 h, the animals were killed, and gastric lesions in the mucosa (54) were scored and expressed as ulcer index as follows: 0 = no pathology; 1 = one pinhead ulcer. The sum of the total scores divided by the number of animals gives the ulcer index.

**Pylorus Ligation-induced Gastric Acid Secretion—** Hypersecretion was induced in rats by pylorus ligation (55) under light anesthesia with ketamin (12 mg/kg of body weight). The animals were killed 2.5 h after ligation, and the clarified gastric fluid volume was determined and titrated for acid content with 1 mm NaOH (13).
DNA was isolated from the lysate by a phenol-chloroform extraction containing 0.9% saline and 20 mM EDTA, pH 8, washed twice with ethyl acetate (1:1), dissolved in 0.6 ml of a solution containing 6 M trifluoroacetic acid, and centrifuged, and the supernatant was used for onset of restraint cold stress. The third group received omeprazole (8 mg/kg) pretreated stressed rats was homogenized in 50 mM sodium phosphate buffer, pH 7.4, in a Potter-Elvehjem glass homogenizer for 45 s to get 10% homogenate. After centrifugation at 600 × g for 10 min, the proteins from 0.8 ml of the supernatant were precipitated with 5% trichloroacetic acid and allowed to react with 0.5 ml of 10 mM 2,4-dinitrophenylhydrazine for 1 h. After precipitation with 10% trichloroacetic acid, the protein was washed thrice with a mixture of ethanol-ethyl acetate (1:1), dissolved in 0.6 ml of a solution containing 6 M guanidine HCl in 1 M potassium phosphate adjusted to pH 2.3 with trifiuoracetic acid, and centrifuged, and the supernatant was used for measurement of carbonyl content at 362 nm (ε = 22000 M⁻¹ cm⁻¹).

Measurement of Endogenous OH—Hydroxy radical generated in the gastric mucosa was measured using Me₃SO as ‘OH scavenger (58–60). Briefly, the control group was kept at room temperature without any stress after administration (intraperitoneally) of 1 ml of Me₃SO. The second group received the same amount of Me₃SO 30 min before the onset of restraint cold stress. The third group received omeprazole (8 mg/kg intraperitoneally) 30 min prior to Me₃SO administration and were then subjected to stress. After 3.5 h of stress, the animals were killed, and fundic stomach was processed for the extraction of methanethiol and sulfenic acid. Me₃SO with Me₂SO was allowed to react with the Fast Blue BB salt to yield an yellow chromophore that was measured at 425 nm using benzene-sulfinic acid as standard.

Measurement of DNA Damage in Vivo as an Index of Apoptosis—To study DNA fragmentation as an index of apoptosis, DNA was isolated from fundic mucosal surface epithelial cells of normal rats and rats subjected to restraint cold stress without or after pretreatment with omeprazole (8 mg/kg). Fundic mucosa (~1.5 g) from three animals was scraped, minced separately in PBS-E (50 mM sodium phosphate buffer containing 0.9% saline and 20 mM EDTA, pH 8), washed twice with PBS-E, and finally suspended in 2 ml of PBS-E containing 0.5 mM EDTA. The suspension was incubated at 37 °C for 1 h, followed by the addition of Pronase E (1 mg/ml), and further incubated for 15 min at 37 °C. It was centrifuged at 1000 rpm for 5 min. The pellet was dispersed and incubated with 2 ml of a lysis buffer containing 50 mM Tris-Cl, pH 8, 20 mM EDTA, 10 mM NaCl, and 1% w/v SDS for 15 min. It was centrifuged at 14,000 × g for 15 min, and DNA was isolated from the lysate by a phenol-chloroform extraction procedure (61). DNA was dissolved in 10 mM Tris-Cl, pH 8, containing 1 mM EDTA by gentle shaking at 65 °C. Residual contaminating RNA was removed by incubating the DNA solution with 1 μg/ml DNase-free RNase at 37 °C for 1 h followed by 0.1 mg/ml proteinase K for 3 h. Phenol-chloroform extraction was repeated to obtain purified DNA that was digested and separated by electrophoresis at 10000 g for 1 h. DNA was dissolved in 20 μl of TE buffer (10 mM Tris-Cl, pH 8.0, 25 mM EDTA, 0.5% SDS, 0.1 μg/ml proteinase K, and 1 μg/ml RNase) by incubating in a shaker bath at 52 °C for 15 h. DNA was extracted from the lysate after phenol-chloroform extraction as described (61). Rat DNA (~200 ng) or human DNA (~300 ng) was incubated in a ‘OH-generating system containing 100 mM sodium phosphate buffer, pH 7.4, 0.2 mM CuSO₄, and 1 mM ascorbate (59) in a total volume of 30 μl for a period of 30 min at 37 °C in presence or absence of omeprazole or lansoprazole. The reaction was stopped by the addition of 1 μl of catalase, and electrophoresis was carried out in a 2% agarose gel.

Scavenging of ‘OH by Lansoprazole—Hydroxyl radical was generated in vitro in the Cu²⁺-ascorbate system (59, 60) and quantitated as described by Babbs and Steiner (58). The assay system contained in a final volume of 1 ml: 50 mM sodium phosphate buffer, pH 7.4, 0.2 mM CuCl₂, 2 mM ascorbate, and 2 mM MeSO in the absence and presence of lansoprazole. After incubation at 37 °C for 1 h, the reaction was stopped with 0.5 mM EDTA, and the methanesulfonic acid formed was extracted and allowed to react with Fast Blue BB salt for quantitation as described (58).

Isolation of ‘OH Mediated Oxidation Product of Lansoprazole—Because crystalline lansoprazole is readily available commercially, this experiment was carried out with lansoprazole instead of omeprazole with the aim of isolating the ‘OH-mediated oxidation product of lansoprazole, if the latter scavenges ‘OH. Lansoprazole (0.2 mM) was incubated at 37 °C for 3.5 h with 0.2 mM CuCl₂ and 2 mM ascorbate in the presence of 10 mM phosphate buffer, pH 7.4, in a final volume of 400 ml. The content was evaporated in a Eyela N-N series rotary vacuum evaporator, and the residue was extracted repeatedly with chloroform followed by methanol. A control system containing 0.2 mM lansoprazole in 10 mM phosphate buffer was incubated under similar conditions without a ‘OH-generating system and subjected to the same extraction procedure to find out whether any aerial oxidation occurs or not. The major oxidation product was isolated from the methanol extract after separation by preparative TLC on plate (8 × 18 cm) coated with silica gel 60 F₂₅₄ using chloroform:methanol (90:10) as the mobile phase. The compounds were detected by spraying with iodine vapor. The major oxidation product was recovered from the TLC plate by elution with methanol and was further purified by Waters HPLC system using Waters 15 μ 100 A Deltapak-C₁₈ semipreparative column (7.8 × 300 mm) eluted with methanol:water (80:20) at a flow rate of 1 ml/min. The absorbance was monitored at 285 nm.

Mechanism of Antiulcer Effect of Omeprazole—The HPLC-purified major oxidation product of lansoprazole was dissolved in CDC₁₈, and the ¹H NMR spectrum was recorded in a Bruker 300 MHz NMR spectrometer. Molecular weight was determined by electron impact (EI⁺) mass spectrometry using Jeol JMS 600 mass spectrometer. The UV-visible spectrum was recorded in a Shimadzu UV-1601 spectrophotometer.

Statistical Analysis—All of the data were expressed as the means ± S.E. The significance was calculated using a Student’s t test.

RESULTS

Differential Effect of Omeprazole in Blocking Gastric Ulcer and Gastric Acid Secretion—To investigate whether omeprazole blocks gastric lesions through an independent mechanism other than the inhibition of acid secretion, the dose-dependent effect of omeprazole was studied both on stress- and indomethacin-induced gastric ulceration and pylorus ligation-induced gastric acid secretion. Fig. 2A indicates that omeprazole dose-dependently blocks both stress and indomethacin-induced gastric lesions showing nearly 90% inhibition at 8 and 16 mg/kg, respectively. More than 90% of the animals showed no gastric lesion at all. In contrast, omeprazole blocks pylorus ligation-induced acid secretion at a higher dose, causing nearly 90% inhibition at 20 mg/kg (Fig. 2B). However, one significant finding is evident from the dose-response patterns for blocking gastric damage and acid secretion. At the dose of 2.5 mg/kg, omeprazole cannot block acid secretion at all, whereas at a slightly lower dose of 2 mg/kg, omeprazole blocks stress ulcer by 70%, and indomethacin ulcer by 50%. The efficacy of omeprazole in blocking gastric damage and acid secretion can be more accurately determined by the potency (ED₅₀) calculation from the dose-response profiles. Whereas the ED₅₀ values for inhibiting stress and indomethacin-induced gastric lesions are 0.8 and 2 mg/kg, respectively, that for the induced acid secretion is 3.25 mg/kg. The data indicate that omeprazole blocks gastric lesions through a mechanism independent of its role on acid secretion. The relationship between gastroprotection and
Acid inhibition by omeprazole has been further clarified from the time course studies of inhibition as shown in Table I. The results indicate that at the initial period of 1 h when acid secretion is inhibited by 50% only, gastroprotection by omeprazole is almost complete, showing around 90% inhibition of the gastric lesions caused by stress or indomethacin. At later time periods of 2.5 and 3.5 h, gastroprotection remains more or less at the same level when acid inhibition is increased to 80%. It is thus clear that omeprazole can offer gastroprotection almost completely even when it cannot completely block acid secretion.

In other words, omeprazole-induced gastroprotection is not decreased with relatively higher rate of acid secretion. Omeprazole thus protects gastric lesions through mechanisms other than acid inhibition.

Role of Hydroxyl Radical on Gastric Ulceration—To assess whether endogenous 'OH plays any significant role on the development of gastric lesions, the effect of Me₃SO, a specific 'OH scavenger (58) was studied on both stress- and indomethacin-induced gastric lesions. The data indicate that Me₃SO causes 87% protection of stress ulcer (Fig. 3A) and 94% protection of indomethacin ulcer (Fig. 3B) without affecting the luminal acid content. The results indicate that 'OH plays one of the major roles in stress- or indomethacin-induced gastric lesions. Fig. 4 further shows that time-dependent severity of gastric lesions (ulcer index) correlates well with the increased generation of 'OH and not with the luminal acid content, suggesting that 'OH plays a significant role in the gastric damage.

Effect of Omeprazole on 'OH-mediated Oxidative Damage of the Gastric Mucosa—Because 'OH is one of the major causative factors for gastric ulceration and creates oxidative damage by increased membrane lipid peroxidation and protein oxidation (22), the effect of omeprazole was therefore studied on these two parameters. Table II shows that omeprazole blocks stress-induced generation of 'OH and at the same time completely prevents radical-induced increased lipid peroxidation and protein oxidation. Omeprazole thus blocks gastric oxidative damage by acting as an antioxidant through scavenging of endogenous 'OH. The potency of omeprazole as an antioxidant to block gastric lesions was compared with some natural and synthetic antioxidants having antilucer activity. The dose-response profiles clearly indicate that omeprazole (Fig. 5A) is more potent than the naturally occurring antioxidants such as vitamin E or melatonin (Fig. 5B) or some synthetic antioxidants such as desferrioxamine (Fig. 5C), a transition metal ion.
Mechanism of Antiulcer Effect of Omeprazole

TABLE II

<table>
<thead>
<tr>
<th>OH</th>
<th>Lipid peroxidation</th>
<th>Protein carbonyl</th>
</tr>
</thead>
<tbody>
<tr>
<td>nmol/g</td>
<td>nmol TBARS/mg protein</td>
<td>nmol/mg protein</td>
</tr>
<tr>
<td>Control</td>
<td>0.69 ± 0.05</td>
<td>1.14 ± 0.19</td>
</tr>
</tbody>
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| Control + stress | 194 ± 27 | 1.66 ± 0.20
| Control + omeprazole + stress | 48 ± 28 | 0.64 ± 0.09 |

*a* p < 0.01 versus control. 
*b* p < 0.001 versus control and control + stress. 
*p* p < 0.05 versus stress.

Effect of Omeprazole on endogenous hydroxyl radical and oxidative damage as measured by lipid peroxidation and protein oxidation. Omeprazole was injected (intraperitoneally) 30 min before the onset of restraint cold stress. Lipid peroxidation, protein oxidation, and OH were measured as described in the text. The data for OH were presented after subtracting the control value, which is 39 ± 9 nmol/g. TBARS, thiobarbituric acid-reactive substance.

Chelator to prevent 'OH generation, or PBN (Fig. 5D), a radical scavenger.

Effect of Omeprazole on DNA Damage of the Mucosal Cell as an Indication of Apoptosis—Apoptotic cell death is associated with DNA fragmentation, and oxidative attack is thought to be one of the underlying mechanisms. Because restraint cold stress causes extensive damage of the surface epithelium with numerous cells sloughed off into the gastric lumen because of cell death (13), it is interesting to investigate whether this process is associated with apoptotic cell death or not. Fig. 6 shows that stress-induced gastric epithelial cell damage is associated with DNA fragmentation showing typical DNA ladder (lane 2), an index of cell apoptosis. However, omeprazole pretreatment can completely block stress-induced DNA fragmentation (lane 3), suggesting its antiapoptotic role to prevent cell death during ulceration.

Antioxidant Role of Omeprazole and Lansoprazole in Blocking Oxidative DNA Damage in Vitro—Oxidative damage of DNA can be studied in vitro when incubated in a 'OH-generating system. Fig. 7A shows that rat gastric mucosal cell DNA (lane 1), when incubated in the Cu^{2+}-ascorbate-mediated 'OH-generating system, is completely fragmented into small pieces so that the main DNA band (lane 1) is not observed at all in lane 2. DNA can be completely protected from the oxidative damage by catalase (lane 7), suggesting the involvement of H_2O_2 in the process. Protection is also evident with the spin trap DMPO (lane 8), suggesting the generation of the radical species. The data indicate that DNA is oxidatively damaged by 'OH generated from H_2O_2 in presence of Cu^{2+} and ascorbate (reducing equivalent of O_2^) through a metal-catalyzed Haber-Weiss reaction (28). However, omeprazole blocks this oxidative damage in a concentration-dependent manner (lanes 3–6), showing complete protection at 500 μM. Fig. 7B shows similar effect of lansoprazole on the protection (lanes 3–5) of oxidative damage of DNA. Lansoprazole completely prevents oxidative damage at 500 μM (lane 5). Lanes 6 and 7 show the protective effects of catalase and DMPO, respectively. Lansoprazole can also block oxidative damage of human gastric mucosal DNA (Fig. 7C) in a concentration-dependent manner (lanes 3–6), showing complete protection at 500 μM (lane 6).
absence and presence of lansoprazole. Fig. 8 indicates that lansoprazole can directly decrease the level of \( \text{OH} \) in a concentration-dependent manner showing 90\% inhibition at 2 mM. Because lansoprazole does not decompose \( \text{H}_2\text{O}_2 \) or chelate \( \text{Cu}^{2+} \) (data not shown), the effect is not due to the decreased generation of \( \text{OH} \) from endogenous \( \text{H}_2\text{O}_2 \) through metal-catalyzed Haber-Weiss reaction.

Identification and Isolation of Oxidation Product of Lansoprazole—To investigate the possibility for scavenging of \( \text{OH} \) by lansoprazole to form an oxidation product, lansoprazole was incubated in the \( \text{Cu}^{2+} \)-ascorbate system, and the mixture after extraction with chloroform followed by methanol was subjected to TLC (Fig. 9A). Lane 1 shows the single spot of commercial lansoprazole used in this study. Lane 2, on the other hand, shows the formation of at least four oxidation products of lansoprazole, of which spot 4 is the major oxidation product. None of these products were detected when lansoprazole was incubated only in phosphate buffer (not shown). This major oxidation product was isolated from the preparative TLC plate and subjected to HPLC (Fig. 9B). The chromatogram (Fig. 9B, tracing 1) shows a major peak of the product preceded by a number of small peaks probably contributed by some impurities from the silica gel. These impurities were removed by isolating the compound in the major peak by HPLC. The HPLC-purified product shows more than 95\% purity as evidenced from both these studies that omeprazole offers gastroprotection through a mechanism other than acid inhibition. Second, evidence has been provided to show that majority of the gastric lesions is caused by endogenous \( \text{OH} \), as revealed by almost complete (~90\%) protection by \( \text{Me}_2\text{SO} \), a specific \( \text{OH} \) scavenger (5b). This is further supported by the finding that time-dependent severity of gastric lesions correlates well with the increase in endogenous \( \text{OH} \) and has no correlation with the luminal acid content. Almost complete protection by other antioxidants like melatonin, desferrioxamine, and PBN further strengthens the view that \( \text{OH} \) plays one of the major roles in the development of gastric lesions. Third, omeprazole scavenges the endogenous \( \text{OH} \) and thus blocks radical-induced oxidative damage of the membrane lipid and proteins. Fourth, DNA damage and fragmentation, an indication of apoptotic cell death during ulceration, is also protected by omeprazole. Omeprazole or lansoprazole also protects \( \text{OH} \)-mediated oxidative damage of DNA in vitro. These studies indicate that omeprazole blocks gastric lesions by acting as an antioxidant and antiapoptotic compound. Finally, using lansoprazole as an analogue of omeprazole, evidence has been provided to show that lansoprazole scavenges \( \text{OH} \) to form lansoprazole sulfone as a major oxidation product.

Gastric mucosal integrity is maintained by a dynamic process of cell death and cell proliferation. Among various factors involved in gastric mucosal lesions, oxidative damage (13, 22, 25–37) and apoptotic cell death (39–51) play significant roles in the loss of gastric mucosal integrity caused by various aggres-

**DISCUSSION**

The salient points of the present studies are that omeprazole blocks stress and indomethacin-induced gastric lesions through mechanism independent of its role on acid secretion. Omeprazole can protect ulcer at a dose that does not inhibit acid secretion. Time course studies on gastroprotection and acid inhibition further indicate that omeprazole can almost completely block gastric lesions at the initial period when acid secretion is not completely inhibited. On the other hand, Larsen and co-workers (18, 53) showed that intravenous doses of omeprazole that block acid secretion cannot protect ethanol-induced gastric lesions, suggesting that acid inhibition plays no significant role on gastroprotective effect of omeprazole, which is observed after oral administration of the drug, presumably through its local action. Although this observation is opposite to ours because of the different experimental design and different models of ulcer and acid secretion used, nevertheless, it is clear from both these studies that omeprazole offers gastroprotection through a mechanism other than acid inhibition.
sive factors. In other words, lesions develop when oxidative damage and apoptosis predominate over the healing process by cell proliferation where the role of various growth factors, nitric oxide, endothelin, angiogenesis, mitogen-activated protein kinases, and oncogene (c-myc, c-Ha-ras, and c-fos) expression has been demonstrated. The modern approach of understanding the mechanism of the antiulcer effect of omeprazole should therefore be directed toward exploring its plausible role in preventing oxidative damage and apoptosis as well as on the promotion of healing process by cell proliferation. As far our knowledge goes, this is the first evidence to show that omeprazole blocks gastric lesions by preventing oxidative damage of the gastric mucosal cells. Although omeprazole blocks ulceration at a lower dose (≤2.5 mg/kg) without inhibiting acid secretion, suggesting its independent antiulcer activity, at higher doses its additional antisecretory action definitely exerts beneficial effect by preventing aggravation of the wound, thereby helping the healing process by cell proliferation. The question arises as to how omeprazole offers antiulcer activity independent of acid secretion. Our studies indicate that omeprazole is highly effective in blocking membrane lipid peroxidation and protein oxidation, which occur because of oxidative damage by ROS especially by 'OH (13, 22, 25–37). Omeprazole can scavenge the endogenous 'OH and thus prevents oxidative damage and gastric lesions. By blocking oxidative damage through lipid peroxidation and protein oxidation, omeprazole prevents loss of membrane permeability and dysfunction of the cellular proteins, leading to survival of the functionally active cells. Many natural and synthetic compounds are known to offer antiulcer effect by acting as antioxidants. Melatonin (a pineal hormone), vitamin E, PBN, or desferrioxamine directly or indirectly decreases the endogenous level of 'OH to block gastric ulcer (22, 59). Comparative bioefficacy studies indicate that omeprazole is superior to these antioxidants in blocking gastric lesions. However, the most important effect of omeprazole lies in its novel antiapoptotic role during ulceration, as evidenced by prevention of DNA fragmentation in vivo. Apoptosis of mucosal cells occurs almost in all types of gastric ulcer (39–51) where DNA damage and fragmentation occur by various aggressive factors (39, 41, 45–47). Using histological section and terminal deoxynucleotidyltransferase biotin-dUTP nick end labeling (TUNEL) staining technique (39), gastric mucosal cell apoptosis was detected up to 4 h after stress, following which cell proliferation was found to be significantly increased to promote mucosal healing (39). Moreover, apoptosis is triggered by the up-regulation of apoptosis-promoting Bax mRNA and down-regulation of the antiapoptotic Bcl-2 mRNA expression (39). We have, however, directly demonstrated stress-induced DNA fragmentation in the surface epithelial cell and the beneficial role of omeprazole to block it, thereby preventing apoptotic cell death and gastric lesions. It is not clear yet how differential expression of Bax and Bcl-2 proteins controls apoptosis. However, decreased gastric mucosal blood flow (39) leads to the ischemic condition to generate ROS through alteration of antioxidant systems of gastric mucosa (13, 22), which may cause apoptosis through oxidative damage of DNA (39, 45, 46). However, ischemia may also cause apoptosis through other mechanisms such as involvement of Bcl-2, Bax, and c-Fos proteins (74, 75). Excessive
Mechanism of Antilucler Effect of Omeprazole

generation of nitric oxide by gastric mucosal-inducible nitric oxide synthase by stress also promotes apoptosis through increased formation of ROS (76, 77). That ROS can cause oxidative damage of DNA isolated from both rat and human gastric mucosal epithelial cells has been evident from our in vitro studies where incubation of DNA with an ‘OH-generating system (22, 59, 78) causes extensive DNA degradation, which is sensitive to catalase and DMPO. Both omeprazole and lansoprazole have a unique capacity to block this oxidative damage, indicating its potent antioxidant role to protect DNA from the attack of ‘OH. This could be achieved if omeprazole or lansoprazole can directly scavenge the ‘OH to form oxidation product. Lansoprazole when incubated in the ‘OH-generating system, can in fact diminish the level of ‘OH by its direct scavenging action. This is evident by the observation that incubation of one molecule of water.

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