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

Kaushik Biswas, Uday Bandyopadhyay, Ishita Chattopadhyay, Archana Varadaraj, Esahak Ali, and Ranajit K. Banerjee

From the Departments of Physiology and Department of Immunology, Indian Institute of Chemical Biology, 4, Raja S. C. Mullick Road, Kolkata 700 032, India

ABSTRACT

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 lesions, 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 385, which is 16 mass units higher than that of lansoprazole (m/z 369). 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.

INTRODUCTION

Proton pump inhibitors such as omeprazole, lansoprazole, pantoprazole, and rabeprazole are extensively used for therapeutic 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. These compounds share a common structural motif contributed by a substituted pyridyl-methylsulfanyl benzimidazole. Inhibition of gastric acid secretion by these compounds is considered to be an important step to control the disorders. Proton pump inhibitors inhibit acid secretion by irreversibly interacting with the H⁺-K⁺-ATPase, the terminal proton pump of the parietal cell. 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. Although omeprazole, the primary member of the proton pump inhibitors, has been extensively used to control these disorders, lansoprazole, the second member of the substituted benzimidazole containing a trifluoroethoxy group, has also been used more recently.

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. 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, 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. Suppression of intragastric acid also helps in the healing of ulcer. 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. Restraint cold stress or restraint water immersion stress induces gastric lesions, which are associated with a decreased or normal level of acid secretion. Because restraint or water immersion stress significantly decreases acid secretion induced by pylorus ligation, acid plays a minor role in stress ulcer. Administration of antacids to neutralize secreted acid does not protect stress ulcer, 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. Because acidity as high as 0.6 M HCl can experimentally produce gastric lesions, mild irritants like 0.35 M HCl prevents gastric damage caused by stronger necrotizing agent through "adaptive cytoprotection" mediated by increased formation of prostaglandin.

It is now generally agreed that gastric

lesions develop when the delicate balance between some gastroprotective and aggressive factors is lost. Although the cellular and molecular basis of gastric mucosal defense against gastrodamaging factors are known, the mechanism of mucosal damage by the aggressive factors is not fully clear today. Stress, nonsteroidal antiinflammatory drugs, and H. pylori cause mucosal damage through a number of mechanisms, of which some reactive oxygen species (ROS) such as O and OH are now considered to be one of the major causative factors for mucosal lesions through oxidative damage. Lipid peroxidation, an important parameter for ·OH-induced oxidative damage of membrane, is increased in gastric lesions caused by ethanol, indomethacin, and water immersion stress. Increased lipid peroxidation, increased protein oxidation, and decreased glutathione level are also evident in restraint cold stress-induced gastric lesions as a result of oxidative damage caused by the significant generation of OH. Hydroxyl radical-mediated oxidative damage of membrane lipid and protein and depletion of glutathione have also recently been reported in human gastric ulcer. Hydroxyl radical is generated from O and H₂O₂ in presence of a trace amount of transition metal (released from protein by acidosis caused by

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Omeprazole for severe reflux esophagitis in children.

De Giacomo C, Bawa P, Franceschi M, Lunetti O, Fiocca R. Clinica Pediatrica, Università di Pavia, IRCCS Policlinico, S. Matteo, Italy.

BACKGROUND:

Severe esophagitis is a rare complication of gastroesophageal reflux in children. In adults, omeprazole therapy of severe erosive esophagitis has become the gold standard short-term treatment of the disease. In children, data on its use are limited, and problems about the dosage are unresolved. The aim of this study was to evaluate the efficacy of a simplified, body-weight-based daily dosage of omeprazole in children with severe esophagitis.

METHODS:

Ten children (median age 75.6 months; range 25-109 months) with severe esophagitis were prospectively investigated. All patients were evaluated by endoscopy, histology, and 24-h pH-metry study before and after 3 months of omeprazole. The starting dose of omeprazole was 20 mg as a single daily dose in children weighing less than 30 kg, and 40 mg daily for those weighing over 30 kg.

RESULTS:

A significant improvement in all the children was demonstrated after 3 months of treatment by clinical, endoscopic, and pH-metry assessment. However, histologic study failed to show significant improvement of both inflammatory and hyperplastic findings. Relapse occurred in six of 10 patients after discontinuation of therapy.

CONCLUSIONS:

Omeprazole is effective in the short-term treatment of severe esophagitis in children. The daily dose of the drug could be easily based on the body weight. The persistence of histologic features of esophagitis in spite of clinical and endoscopic healing could be an indicator of poor outcome.

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ischemia) by Haber-Weiss reaction (38) following alteration of the antioxidant enzymes of the gastric mucosa. Recent studies also indicate that programmed cell death or apoptosis plays a significant role in gastric ulceration. Gastric mucosal lesions caused by stress, indomethacin, ethanol, and *H. pylori* are also due to increased cell death by apoptosis. Apoptosis is promoted because of an imbalance between the Bcl-2 family of antiapoptotic protein and apoptotic Bax protein in stress ulcer. Induction of tumor necrosis factor- α and release of cytochrome c to activate caspase-3-like protease are involved in apoptotic cell death in indomethacin ulcer. Apoptosis also occurs because of nitric oxide production through induction of nitric-oxide synthase by *H. pylori*. Involvement of ROS and oxidative damage of DNA and DNA fragmentation have also been evident in apoptotic cell death in gastric mucosal injury.

Although omeprazole is believed to offer its antiulcer activity through acid suppression by inactivating the $H^+-K^+-ATPase$, very little is known regarding its role in 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, 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. 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 $\cdot OH$ plays one of the major roles in gastric lesions and that omeprazole acts as a potent antioxidant to scavenge the endogenous $\cdot OH$, 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 lansoprazole blocks $\cdot OH$ -induced oxidative damage of DNA by scavenging $\cdot OH$ in vitro. Analysis of the major oxidation product of lansoprazole indicates that this antioxidant activity is due to scavenging of $\cdot OH$ to form an oxygenated product that is assigned to lansoprazole 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-tert-butyl nitron (PBN), thiobarbituric acid, ethidium bromide, ascorbic acid, 2,4-dinitrophenylhydrazine, collagenase type 1A, Pronase E, proteinase K, RNase, catalase, guanidine HCl, Fast Blue BB salt, tetraethoxypropane, benzenesulfonic 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), Me2SO, and TLC plates coated with silica gel 60 F254 were procured from Merck.

Animals Used-- Sprague-Dawley rats (200-250g) 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 ($4 \pm 1^\circ C$) stress for 3.5 h. 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. Luminal acid content was determined by titration with 1 mM NaOH using an autoburette pH stat system from Radiometer (Copenhagen, Denmark).

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 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 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.

Measurement of Lipid Peroxidation as an Index of Oxidative Damage-- Lipid peroxidation products of the mitochondrial membrane fraction of fundic stomach homogenate were determined as thiobarbituric acid-reactive substances. The fundic stomach from control, stress-ulcerated, and omeprazole (4 mg/kg)-pretreated stressed rats was homogenized in ice-cold 0.9% saline in a Potter-Elvehjem glass homogenizer for 45 s to get 5% homogenate. One ml of the mitochondrial membrane fraction obtained after differential centrifugation was allowed to react with 2 ml of trichloroacetic acid-thiobarbituric acid-HCl reagent containing 0.01% butylated hydroxytoluene, heated in a boiling water bath for 15 min, cooled, and centrifuged, and the supernatant was used for thiobarbituric acid-reactive substance determination at 535 nm using tetraethoxypropane as standard.

Measurement of Protein Carbonyl Content as an Index of Oxidative Damage-- Protein oxidation was measured as carbonyl content in the low speed super-

natant of the fundic stomach homogenate. The fundic stomach from control, stress-ulcerated, and 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 $600 \times g$ 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 20 mM potassium phosphate adjusted to pH 2.3 with trifluoroacetic acid, and centrifuged, and the supernatant was used for measurement of carbonyl content at 362 nm ($\epsilon = 22000 M^{-1} cm^{-1}$).

Measurement of Endogenous $\cdot OH$ -- Hydroxyl radical generated in the gastric mucosa was measured using Me2SO as $\cdot OH$ scavenger. Briefly, the control group was kept at room temperature without any stress after administration (intraperitoneally) of 1 ml of Me2SO. The second group received the same amount of Me2SO 30 min before the onset of restraint cold stress. The third group received omeprazole (8 mg/kg intraperitoneally) 30 min prior to Me2SO 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 methanesulfonic acid formed by reaction of $\cdot OH$ with Me2SO. Methanesulfonic acid was allowed to react with the Fast Blue BB salt to yield an yellow chromophore that was measured at 425 nm using benzenesulfonic 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 mg/ml collagenase. The suspension was incubated at $37^\circ C$ for 1 h with stirring, followed by the addition of Pronase E (1 mg/ml), and further incubated for 15 min at $37^\circ C$. 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 $14,000 \times g$ for 15 min, and DNA was isolated from the lysate by a phenol-chloroform extraction procedure. DNA was dissolved in 10 mM Tris-Cl, pH 8, containing 1 mM EDTA by gentle shaking at $65^\circ C$. Residual contaminating RNA was removed by incubating the DNA solution with $1 \mu g/ml$ DNase-free RNase at $37^\circ C$ for 1 h followed by 0.1 mg/ml proteinase K for 3 h.

The safety of omeprazole during pregnancy: a multicenter prospective controlled study.

Lalkin A, Loeblein R, Addis A, Ramezani-Namin F, Mastrolacovo P, Mazzone T, Vial T, Bonati M, Koren G. *Maternal program, Toronto, Ontario, Canada.*

OBJECTIVES:

Our purpose was to determine whether omeprazole use during pregnancy is associated with an increased risk of malformations, spontaneous abortions, decreased birth weight, or perinatal complications.

STUDY DESIGN:

In a multicenter, prospective controlled study, pregnant women exposed to omeprazole during gestation were matched with controls exposed to non-teratogens and with disease-paired controls who used histamine blockers for similar indications. The primary end point was the incidence of major malformations.

RESULTS:

One hundred thirteen pregnant women were exposed to omeprazole during pregnancy. Rates of major malformations in the omeprazole group (4%) did not differ from controls exposed to non-teratogens (2%) ($P = .68$, relative risk = 1.94, 95% confidence interval 0.36 to 10.36) and disease-paired controls (2.8%). Birth weight, gestational age at delivery, preterm deliveries, and neonatal complications were comparable among the three groups.

CONCLUSIONS:

No association was found between exposure to omeprazole during the period of organogenesis and increased risk for major malformations. Exposure throughout pregnancy is not associated with increased risk of spontaneous abortions, decreased birth weight, or perinatal complications.

Phenol-chloroform extraction was repeated to obtain purified DNA that was dissolved in 10 mM Tris-Cl buffer, pH 8, containing 1 mM EDTA. To study DNA fragmentation, DNA was loaded on to a 1.5% agarose gel. Electrophoresis was carried out at 100 V for 1.5 h in TBE (90 mM Tris borate, 2 mM EDTA, pH 8) buffer, and DNA was visualized by UV exposure after staining with ethidium bromide.

Measurement of Reactive Oxygen Species Mediated Oxidative Damage of DNA in Vitro and Its Protection by Omeprazole or Lansoprazole-- To study the antioxidant effect of omeprazole or lansoprazole, ·OH-mediated oxidative damage of DNA isolated from rat mucosal surface epithelial cells or from human gastric mucosal biopsy specimen was studied in absence or presence of omeprazole or lansoprazole. For isolation of human gastric mucosal DNA, the minced mucosa (1 g) was digested with 12 ml of the digestion buffer (100 mM NaCl, 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. 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 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 µg 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 and quantitated as described by Babbs and Steiner. 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 Me₂SO 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 methanesulfinic acid formed was extracted and allowed to react with Fast Blue BB salt for quantitation as described.

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 µm 100 Å 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.

Analysis of Oxidation Product of Lansoprazole-- The HPLC-purified major

oxidation product of lansoprazole was dissolved in CDCI₃, 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. 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. 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. 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 Me₂SO, a specific OH scavenger was studied on both stress- and indomethacin-induced gastric lesions. The data indicate that Me₂SO causes

Does gastro-esophageal reflux provoke the myocardial ischemia in patients with CAD ?

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Slawomir Dobrzycki, Andrzej Banikiewicz, Janusz Korecki, Hanna Bachdzewska-Gajewska, Przemyslaw Prokopcuka, Włodzisław Musiał, Karol A. Kaminski and Andrzej Dobrowicki
Department of Invasive Cardiology, Medical University in Białystok, Ul. Skłodowskiej 24a, 15-276 Białystok, Poland
Department of Gastroenterology, Medical University, Białystok, Poland
Department of Cardiology, Medical University, Białystok, Poland

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Background

Gastro-esophageal reflux disease (GERD) may cause chest pain. The aim was to determine the correlation between ischemia and gastro-esophageal reflux in patients with CAD and to assess the influence of short-term "anti-reflux" therapy on the ischemia in patients with GERD and CAD.

Methods

Fifty patients with angiographically proven CAD underwent simultaneous 24-h continuous ECG and esophageal pH monitoring. We assessed the number of ST-segment depression episodes (ST dep.) and total duration of ischemic episodes, expressed as total ischemic burden (TIB). In pH-metry, we assessed: time percentage of pH lower than 4, total time of pH lower than 4 and the number of pathological refluxes (PR). Patients fulfilling the GERD criteria received a 7-day therapy with omeprazole 20 mg bid. On the 7th day of therapy, simultaneous Holter and esophageal pH monitoring was repeated.

Results

Total number of 224 PRs in 42 patients (84%) was recorded during esophageal pH-metry. GERD criteria were fulfilled in 23 patients (46%). Out of 218 episodes of ST dep., 45 (20.6%) correlated with PR. GERD patients had larger TIB and higher number of ST dep. (p<0.015 and p<0.035, respectively). The anti-reflux therapy reduced all analyzed parameters of esophageal pH monitoring (p<0.0022) as well



Diabetes makes ulcers more deadly

Reuters Health
Friday, April 14, 2006

NEW YORK (Reuters Health) - Among patients with bleeding or perforated ulcers, those with diabetes appear to be at substantially increased risk of dying, according to a report in the journal *Diabetes Care*.

Dr. Reimar W. Thomsen, from Aarhus University Hospital in Denmark, and colleagues hypothesized that diabetic patients with complicated ulcer disease may face a higher short-term death risk because of diabetic blood vessel problems, blurring of symptoms, and an increased risk of overwhelming bacterial infection.

The mortality difference in the perforated ulcer group was even more pronounced. Diabetic patients had a 30-day death rate of 43 percent compared with 24 percent for nondiabetic patients, representing an increased risk of 51 percent.

"Our findings suggest that efforts to improve outcomes from these medical emergencies in diabetic persons should be directed to reducing preventable diabetes complications," the authors conclude.

SOURCE: *Diabetes Care*, April 2006.

87% protection of stress ulcer and 94% protection of indomethacin ulcer without affecting the luminal acid content. The results indicate that OH plays one of the major roles in stress- or indomethacin-induced gastric lesions 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, the effect of omeprazole was therefore studied on these two parameters. 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 antiulcer activity. The dose-response profiles clearly indicate that omeprazole is more potent than the naturally occurring antioxidants such as vitamin E or melatonin or some synthetic antioxidants such as desferrioxamine, a transition metal ion chelator to prevent OH generation, or PBN, 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, it is interesting to investigate whether this process is associated with apoptotic cell death or not. Stress-induced gastric epithelial cell damage is associated with DNA fragmentation showing typical DNA ladder, an index of cell apoptosis. However, omeprazole pretreatment can completely block stress-induced DNA fragmentation, 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. Rat gastric mucosal cell DNA, when incubated in the Cu²⁺-ascorbate-mediated OH-generating system, is completely fragmented into small pieces so that the main DNA band is not observed at all. DNA can be completely protected from the oxidative damage by catalase, suggesting the involvement of H₂O₂ in the process. Protection is also evident with the spin trap DMPO, suggesting the generation of the radical species. The data indicate that DNA is oxidatively damaged by OH generated from H₂O₂ in presence of Cu²⁺ and ascorbate (reducing equivalent of O) through a metal-catalyzed Haber-Weiss reaction. However, omeprazole blocks this oxidative damage in a concentration-dependent manner, showing complete protection at 500 μM. Lansoprazole completely prevents oxidative damage at 500 μM. Lansoprazole can also block oxidative damage of human gastric mucosal DNA in a concentration-dependent manner, showing complete protection at 500 μM.

Effect of Lansoprazole on the Level of OH in Vitro--

The protection of the oxidative

DNA damage by omeprazole or lansoprazole may be explained as being due to its direct scavenging action on OH so that DNA is spared from the radical attack. To study the OH scavenging action, OH generation was measured in vitro in the Cu²⁺-ascorbate system in the absence and presence of lansoprazole. Lansoprazole can directly decrease the level of OH in a concentration-dependent manner showing 90% inhibition at 2 mM. Because lansoprazole does not decompose H₂O₂ or chelate Cu²⁺ (data not shown), the effect is not due to the decreased generation of OH from endogenous H₂O₂ through metal-catalyzed Haber-Weiss reaction.

Identification and Isolation of Oxidation Product of Lansoprazole--

To investigate the possibility for scavenging of OH by lansoprazole to form an oxidation product, lansoprazole was incubated in the Cu²⁺-ascorbate system, and the mixture after extraction with chloroform followed by methanol was subjected to TLC. Single spot of commercial lansoprazole used in this study, 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. This major oxidation product was isolated from the preparative TLC plate and subjected to HPLC. The chromatogram 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 by HPLC profile. The product when run in TLC shows one single spot exactly matching with the R_f value of spot 4.

Characterization of the Oxidation Product of Lansoprazole--

The HPLC-purified oxidation product of lansoprazole when analyzed by EI+ mass spectroscopy shows a clear molecular ion peak at m/z 385, which was 16 mass units higher than that of lansoprazole (m/z 369 not shown). This indicates that the compound is an oxidation product of lansoprazole involv-

ing the addition of one oxygen atom. The oxidation product absorbs at 278 nm because of the presence of the benzene ring. The addition of alkali does not cause any alkaline shift to the higher wavelength characteristic to phenol, indicating that no hydroxylation occurs at the benzene ring to form hydroxylansoprazole. When compared with lansoprazole, no additional aromatic proton signal was detectable in the oxidation product by ¹H NMR (not shown). Thus, the oxidation product having a molecular ion mass of 385 is an oxygenated species of lansoprazole, formed by scavenging of OH.

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, Larsson and co-workers 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. Second, evidence has been provided to show that majority of the gastric lesions is caused by endogenous OH, as revealed by almost complete (~90%) protection by Me₂SO, a specific OH scavenger. This is further supported by the finding that time-dependent severity of gastric lesions correlates well with the increase in endogenous OH and has no correlation with the luminal acid content. Almost

Omeprazole maintenance therapy prevents recurrent ulcer bleeding after surgery for duodenal ulcer

Konstantinos Demertzis, Dimitrios Polymeros, Theodoros Emmanuel, Konstantinos Triantafyllou, Pericles Tassios, Spiros D Ladas

Konstantinos Demertzis, Dimitrios Polymeros, Theodoros Emmanuel, Konstantinos Triantafyllou, Pericles Tassios, Spiros D Ladas, Hepatogastroenterology Unit, 2nd Department of Internal Medicine, "Attikon" University General Hospital, 11528 Athens, Greece Correspondence to: Professor Spiros D Ladas, MD, PhD, Hepatogastroenterology Unit, 2nd Department of Internal Medicine, "Attikon" University General Hospital, Athens, Greece.



To evaluate the omeprazole maintenance therapy in patients with recurrent ulcer bleeding after surgery for duodenal ulcer. We studied 15 consecutive patients with recurrent ulcer bleeding after surgery for duodenal ulcer. Omeprazole (20 mg/d) maintenance therapy was given after ulcer healing. In addition to clinical follow-up, ambulatory 24-h gastric pH assay was performed before and during omeprazole therapy in those patients and controls with previous duodenal ulcer surgery but no ulcer recurrence.

All the 15 ulcers were healed after being treated with omeprazole (40 mg/d) for 2 mo. Eleven patients with two (1-9) episodes of recurrent ulcer bleeding completed the follow-up (43, 12-72 mo). None of them had a bleeding episode while on omeprazole. One patient discontinued the therapy and had recurrent bleeding. The median 24-h fraction time of gastric pH <4 in patients was 80, 46-95%, and was reduced to 32, 13-70% by omeprazole (P=0.002).

Long-term maintenance therapy with omeprazole (20 mg/day) is effective in preventing recurrent ulcer bleeding.

24 Apr 2006

Peptic ulcer incidence declining

Am J Gastroenterol 2006; Early online edition

Results of a Danish population-based epidemiological study have revealed that the incidence of uncomplicated as well as perforated peptic ulcers is decreasing, while the incidence of bleeding ulcers is stable.

The research findings also indicate that an increasing proportion of patients with incident peptic ulcers are using non-steroidal anti-inflammatory drugs (NSAIDs), and that mortality and the risk of complicated ulcer is 2.5- to 37-times higher among patients with incident peptic ulcer than among the general population. The study authors write in the American Journal of Gastroenterology that although the risk of developing a complicated ulcer is known to be increased among individuals with previously diagnosed peptic ulcer, the magnitude of this risk in daily clinical practice is unclear.

Moreover, "while NSAID use is expected to account for an increasing proportion of the peptic ulcers, ecological studies of the relation have shown divergent results," they add.

complete protection by other antioxidants like melatonin, desferrioxamine, and PBN further strengthens the view that OH plays one of the major roles in the development of gastric lesions. Third, omeprazole scavenges the endogenous 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 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 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 aggressive factors. In other words, lesions develop when oxidative damage and apoptosis predominate over the healing process (62) 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 and apoptosis 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. 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. 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 anti-apoptotic role during ulceration, as evidenced by prevention of DNA fragmentation in vivo. Apoptosis of mucosal cells occurs almost in all types of gastric ulcer where DNA damage and fragmentation occur by various aggressive factors. Using histological section and terminal deoxynucleotidyltransferase biotin-dUTP nick end labeling (TUNEL) staining technique, 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. Moreover, apoptosis is triggered by the up-regulation of apoptosis-promoting Bax mRNA and down-regulation of the antiapoptotic Bcl-2 mRNA expression. 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 leads to the ischemic condition to generate ROS through alteration of antioxidant systems of gastric mucosa, which may cause apoptosis through

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 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 lansoprazole in the ·OH-generating system produces four oxidation products, of which the major one shows the addition of 16 mass units (m/z 385) over the mass of lansoprazole (m/z 369), indicating incorporation of an oxygen atom into lansoprazole. Omeprazole and lansoprazole undergo oxidation in cytochrome P-450 systems to produce hydroxyomeprazole or hydroxylansoprazole and omeprazole sulfone or lansoprazole sulfone. Hydroxylation in the benzene ring of lansoprazole in our system does not occur because the oxidation product absorbing at 278 nm does not show the characteristic alkali shift for the formation of phenol. Absence of any additional aromatic proton signal in the

reactive sulfur centered radical intermediate by scavenging the OH, and the intermediate is stabilized to form lansoprazole sulfone by further incorporation of OH at the sulfur radical with the elimination of one molecule of water.

The present study thus provides the first direct evidence for the antioxidant and antiapoptotic role of omeprazole in preventing gastric ulceration by scavenging endogenous OH. This is in contrast to the earlier observation that omeprazole neither protects indomethacin-induced gastric damage nor decreases apoptotic DNA fragmentation. Although a large number of reports (including the present study) indicate that omeprazole prevents indomethacin-induced gastric damage, the inability of omeprazole to block gastric lesions and associated apoptosis is not clear, and hence the conclusion that omeprazole does not possess antiapoptotic property to block indomethacin ulcer remains to be verified. However, a question may arise as to what percentage of total gastroprotection by omeprazole is mediated through block of apoptosis and its antioxidant action by scavenging OH. Because quantitation of total gastroprotection by omeprazole is difficult to assess because it has morphological, cellular, biochemical and pharmacological parameters, extensive studies are required to answer this question. Because gastric ulceration is a multifactorial process, it is possible that gastroprotective effect of omeprazole may partially be mediated through other mechanisms also. Recently omeprazole has been shown to prevent compound 48/80 (mast cell degranulator)-induced gastric lesions (with no acid secretion) by acting as an antiinflammatory agent and also by preventing neutrophil infiltration, activation, and associated mucosal damage. Thus, omeprazole may have multiple modes of action. Although no unifying concept has developed yet on the mechanism of gastric mucosal damage caused by various ulcerogens, it will be interesting to investigate whether omeprazole has a common molecular target for gastroprotection. Because apoptosis and reactive oxygen species play significant roles in mucosal damage, it is conceivable that antiapoptotic and antioxidant role of omeprazole play a major part in the total gastroprotection. These novel actions of omeprazole are of particular clinical significance for the control of **gastroduodenal ulcer by this class of proton pump inhibitors.**

Prevention of stress-related mucosal bleeding with proton-pump inhibitors

Digestive Disease Research Institute, Oklahoma City, OK, USA.
paul-maton@ouhsc.edu

Stress-related gastric mucosal bleeding occurs in a substantial number of critically ill patients, with clinically important gastrointestinal bleeding prolonging intensive care stay and increasing mortality. This paper reviews the role of proton-pump inhibitors in the prevention of stress-related mucosal bleeding. Bleeding prophylaxis appears to be warranted in patients in intensive care units on mechanical ventilation or those who have coagulopathy. Intravenous histamine H2 receptor antagonists, particularly cimetidine, have demonstrated efficacy for the prevention of bleeding in critically ill patients. Standard delayed-release proton-pump inhibitors have not been extensively studied in this patient

group, but there are some data to support their efficacy in increasing intragastric pH, and in the case of intravenous pantoprazole in preventing gastrointestinal bleeding. In a large, randomized controlled trial, immediate-release omeprazole [(R-OME) Zegerid powder for oral suspension; Santarus Inc., San Diego, CA, USA] administered via gastric tube, was as effective as intravenous cimetidine in the prevention of clinically significant bleeding, and more effective in increasing gastric pH. Effective antisecretory therapy does not appear to increase the risk of nosocomial pneumonia. In conclusion, immediate-release omeprazole provides a safe and effective alternative to intravenous cimetidine for the prevention of stress-related mucosal bleeding in critically ill patients.

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oxidative damage of DNA. However, ischemia may also cause apoptosis through other mechanisms such as involvement of Bcl-2, Bax, and c-Fos proteins. Excessive generation of nitric oxide by gastric mucosal-inducible nitric-oxide synthase by stress also promotes apoptosis through increased formation of ROS. That ROS can cause

¹H NMR spectrum also negates the possibility of the formation of phenolic group in the oxidation product. Thus, addition of an oxygen atom has occurred at the sulfur atom of the lansoprazole to form lansoprazole sulfone. The possible mechanism of formation of lansoprazole sulfone from lansoprazole by scavenging OH. Lansoprazole is converted to a highly

Omeprazole maintenance therapy prevents recurrent ulcer bleeding after surgery for duodenal ulcer

Konstantinos Demertzis, Dimitrios Polymeros, Theodoros Emmanuel, Konstantinos Triantafyllou, Pericles Tassios, Spiros D Ladas

Abstract AIM:

To evaluate the omeprazole maintenance therapy in patients with recurrent ulcer bleeding after surgery for duodenal ulcer.

METHODS:

We studied 15 consecutive patients with recurrent ulcer bleeding after surgery for duodenal ulcer. Omeprazole (20 mg/d) maintenance therapy was given after ulcer healing. In addition to clinical follow-up, ambulatory 24-h gastric pH assay was performed before and during omeprazole therapy in those patients and controls with previous duodenal ulcer surgery but no ulcer recurrence.

RESULTS:

All the 15 ulcers were healed after being treated with omeprazole (40 mg/d) for 2 mo. Eleven patients with two (1-9) episodes of recurrent ulcer bleeding completed the follow-up (43, 12-72 mo). None of them had a bleeding episode while on omeprazole. One patient discontinued the therapy and had recurrent bleeding. The median 24-h fraction time of gastric pH < 4 in patients was 80, 46-95%, and was reduced to 32, 13-70% by omeprazole ($p = 0.002$).

CONCLUSION:

Long-term maintenance therapy with omeprazole (20 mg/day) is effective in preventing recurrent ulcer bleeding.

Demertzis K, Polymeros D, Emmanuel T, Triantafyllou K, Tassios P, Ladas SD. Omeprazole maintenance therapy prevents recurrent ulcer bleeding after surgery for duodenal ulcer. *World J Gastroenterol* 2006; 12(5):791-795

<http://www.wjnet.com/1007-9321/12/791.asp>

INTRODUCTION

H pylori eradication therapy and the use of potent proton pump inhibitors (PPIs) have dramatically reduced the need for surgical therapy of peptic ulcer disease. Still, about 10 % of duodenal ulcer patients undergo emergency surgical therapy for acute ulcer bleeding. However, recurrent ulcer is not uncommon as it occurs in 10-15 % of patients after vagotomy and drainage and in 2-5 % of patients after gastric resection. This may be complicated by life threatening acute recurrent ulcer bleeding in certain patients, requiring hospitalization.

Several studies have investigated the rate of ulcer recurrence after duodenal ulcer surgery and the completeness of vagotomy, but only a few studies have evaluated the anastomotic ulcer healing rates after being treated with H2 receptor antagonists (H2RA) or PPI therapy. Studies have shown that *H pylori* infection of the gastric mucosa is not related to ulcer recurrence after gastric surgery. Furthermore, it has been shown that 28 % of anasto-

motric ulcers recur within 6 wk after discontinuing cimetidine therapy, and 33% relapse within a year while on cimetidine maintenance therapy.

These patients are often treated with a second operation. However, to the best of our knowledge, there are no studies investigating the long-term outcome of patients with recurrent post-surgical ulcer and whether maintenance acid suppression therapy with PPIs may prevent recurrent ulceration and/or re-bleeding. Therefore, the present prospective open label study was conducted to investigate gastric pH profile and the effect of omeprazole maintenance therapy in patients presented with recurrent ulcer bleeding after duodenal ulcer surgical therapy.

MATERIALS AND METHODS

Over a 7-year period, this prospective open label study included 15 consecutive male patients admitted to our department due to recurrent acute ulcer bleeding. All patients underwent gastric surgery for duodenal ulcer disease at least 2 years ago.

Clinical study

In each case, emergency endoscopy was performed to confirm recurrent ulcer bleeding. The finding of an ulcer was considered as the bleeding cause if active bleeding or stigmata of recent hemorrhage were noted in the absence of other lesions. The recurrent ulcers were peristomal or duodenal in location. At the same time, detailed history was obtained about the indication and time of past gastric operation and the number of hospital admissions with hematemesis or melena after gastric surgery. History specifically included questions about the use of H2RA, PPIs or non-steroidal anti-inflammatory drugs (NSAIDs), smoking and alcohol abuse. In all the patients fasting serum gastrin and salicylate concentrations were determined to exclude Zollinger-Ellison syndrome and recent consumption of non-steroidal anti-inflammatory drugs. Patients who were on non-steroidal anti-inflammatory drugs were excluded. During endoscopy, multiple gastric mucosal biopsies were obtained to investigate *H pylori* infection.

All patients were initially treated with intravenous omeprazole (20 mg every 12 h) and then orally after discharge from the hospital. *H pylori* eradication therapy was not used to prevent ulcer recurrence, but *H pylori* was eradicated in two patients because of severe *H pylori* gastritis. Follow-up endoscopy was scheduled at 2 mo, while on oral omeprazole (40 mg/d) to confirm ulcer healing. Thereafter, the patients were instructed to receive oral omeprazole (20 mg/d) maintenance therapy, to avoid the use of any non-steroidal anti-inflammatory drugs and to have follow-up every 6 mo as outpatients.

Twenty-four-hour gastric pH studies

Twenty-four-hour gastric pH studies were performed in the following groups on omeprazole therapy (20 mg/d) but not on antiseecretory therapy: patients with first or second degree reflux esophagitis (Los Angeles classification) ("normal" controls); patients with duodenal ulcer; controls who underwent vagotomy and pyloroplasty or gastrojejunostomy for duodenal ulcer but had no ulcer recurrence; controls who had Billroth II partial gastrectomy and patients who had recurrent anastomotic ulcer bleeding after gastric surgery for duodenal ulcer. In the latter group of patients (test group), 24-h gastric pH studies were performed while on omeprazole (40 mg/d, 20 mg/d). Omeprazole was then discontinued and the patients were treated with ranitidine 150 mg twice daily for 2 d, followed by a 2-d washout period before the pH study in patients not on antiseecretory therapy. The duodenal ulcer group included patients admitted to our department for acute ulcer bleeding and volunteered to have 24-h pH studies. The vagotomy and gastrectomy control groups included patients who attended the outpatient clinic for various epigastric symptoms and volunteered to participate in the study after having a negative gastroscopy.

Gastric ambulatory pH monitoring was performed using a monocrystalline anti-

mony pH catheter. The electrode was passed transnasally into the stomach, 10-15 cm below the detectable esophago-gastric junction by endoscopy. The catheter was connected to a portable pH-recording device (Digitrapper Mk III, Synectics Medical AB, Stockholm, Sweden). Recorded data were uploaded into the "Esophogram Analysis Software" for analysis and review. During the 24-h pH studies, patients were encouraged to keep up their usual activities and diet in order to maximize the diagnostic yield of the test. The 24-h fraction (%) time when the gastric pH was below 4 was calculated.

All patients and volunteers gave oral consent after being informed of the purpose of the study by the investigator. The study protocol was approved by the Ethics Committee on Human Studies, Department of Internal Medicine, Medical School of the Athens University. The trial was conducted according to the declarations of Helsinki.

Statistical analysis

We used non-parametric statistics ["Statgraphics-Plus" version 4 for Windows (Manugistics Inc., Rockville, USA)], because of the small number of observations included in each group. Results were presented as median with ranges. We used the non-parametric Mann-Whitney (Wilcoxon) two-sided U-test and the Kruskal-Wallis t-test for two and multiple-sample comparison analysis, respectively. $p < 0.05$ was considered statistically significant.

Asthma and gastroesophageal reflux: acid suppressive therapy improves asthma outcome

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Harding SM, Richter JE, Guzzo MR, Schan CA, Alexander RW, Bradley LA. Department of Medicine, Division of Pulmonary and Critical Care Medicine, University of Alabama, Birmingham, 35294, USA.

PURPOSE: To determine the appropriate omeprazole (Prilosec) dose required for adequate acid suppression in asthmatics with gastroesophageal reflux, whether aggressive acid suppressive therapy of gastroesophageal reflux improves asthma outcome in asthmatics with gastroesophageal reflux, the time course of asthma improvement, and demographic, esophageal, or pulmonary predictors of a positive asthma response to antireflux therapy.

PATIENTS AND METHODS: Thirty nonsmoking adult asthmatics with gastroesophageal reflux (asthma defined by American Thoracic Society criteria and reflux defined by symptoms and abnormal 24-hour esophageal pH testing) were recruited from the outpatient clinics of a 900-bed university hospital. Patients underwent baseline studies including a demographic questionnaire, esophageal manometry, dual-probe 24-hour esophageal pH test, barium esophagram, and pulmonary spirometry. During the 4-week pretherapy phase, patients recorded reflux and asthma symptom scores and peak expiratory flow rates (PEFs) upon awakening, 1 hour after dinner, and at bedtime. Patients began 20 mg/d omeprazole, and the dose was titrated until acid suppression was documented by 24-hour pH test. Patients remained on this acid suppressive dose for 3 months. Responders were identified by a priori definitions: asthma symptom reduction by >20% and/or PEF increase by >20%. Asthma symptom scores, PEF's baseline and posttherapy pulmonary spirometry were analyzed.

RESULTS: Twenty-two (73%) patients were asthma symptom and/or PEF responders: 20 (67%) were asthma symptom responders, and 6 (20%) were PEF responders. Responders reduced their asthma symptoms by 57% ($P < 0.001$), improved their morning and night PEFs by 8% and 9% (both $P < 0.005$), and had improvement in forced expiratory volume at 1 second ($P < 0.02$), mean forced expiratory flow during the middle half (25% to 75%) of the forced vital capacity ($P < 0.04$), and peak expiratory flow ($P < 0.01$) with acid suppressive therapy. Mean acid sup-

RESULTS**Clinical data and follow-up**

Over a 7-year period, 15 consecutive male patients with recurrent ulcer bleeding after surgery for duodenal ulcer were admitted to our department because of acute bleeding episode. Serum gastrin was normal and salicylates were not detected in the serum in any of the 15 patients during their hospitalization. In addition, all patients denied the use of non-aspirin, non-steroidal anti-inflammatory drugs after thorough interrogation.

Ulcer in all the 15 patients was healed after treatment with omeprazole (40 mg/d) for 2 mo. However, four of them did not consent to gastric pH studies and were lost to clinical follow-up within the first year from entering the study. Therefore, data were presented for 11 patients.

Two out of the eleven patients had a history of two gastric operations because of recurrent ulcer bleeding. The time of recurrent ulcer bleeding after gastric surgery was 6 years, averaged 2-18 years. The number of hospital admissions because of post surgical ulcer bleeding was 2, averaged 1-9. At study entry, 3/11 patients were smokers and 5/11 were H pylori-positive. H pylori was successfully eradicated in two of these five patients upon admission to the study. One of the H pylori-negative patients had successful triple eradication therapy at another hospital a year ago.

No recurrent ulcer or re-bleeding occurred during the maintenance therapy (omeprazole 20 mg/d) over the follow-up period (43 mo, averaged 12-72 mo). One patient discontinued treatment and had recurrent bleeding 8 mo after stopping omeprazole. Currently, he was symptom-free on omeprazole maintenance therapy.

There were no significant adverse events related to treatment.

Twenty-four-hour gastric pH studies

Results of the 24-h intragastric pH monitoring studies are shown in Table 2. The "normal" control group included 10 patients with first or second degree reflux esophagitis, who were matched for age, sex and H pylori status with the recurrent post-surgical ulcer patients. They were selected from a pool of patients with reflux esophagitis on 24-h esophageal-gastric pH monitoring. The duodenal ulcer control group included seven patients hospitalized because of duodenal ulcer bleeding. The vagotomy control group included seven patients who had truncal vagotomy and drainage for duodenal ulcer. The gastrectomy control group included seven patients who had partial gastrectomy (Billroth II) for duodenal ulcer. Volunteers of the vagotomy and gastrectomy control groups studied at least 2 years postoperatively had no post-operative ulcer recurrence. The test group included ten of the eleven patients who had truncal vagotomy (n = 5) or Billroth II gastrectomy (n = 5) and recurrent anastomotic ulcer bleeding. Intolerance to nasogastric pH probe was not studied in another patient who had vagotomy and drainage. There were three failures out of 30 24-h gastric pH recordings in the test group. One of the vagotomy controls refused to have the 24-h pH study on omeprazole.

Treatment-free (baseline) 24-hour gastric pH studies

The 24-h gastric pH measurements of the groups studied during treatment-free period are shown in Table 2. There was a statistically significant difference in the 24-h fraction time of gastric pH < 4 among the six groups (T = 29.10, p < 0.001), with the lowest values recorded in the Billroth II gastrectomy control

group. Patients who had vagotomy and recurrent ulcer bleeding had a significantly different (higher) 24-h fraction time of gastric pH < 4 as compared to vagotomy controls (U = 32, p = 0.023). This difference was also significant when bleeding patients after gastrectomy were compared to those of the gastrectomy control group (U = 35, p = 0.006).

Twenty-four hour gastric pH studies (omeprazole 20 mg/day)

Gastric pH studies were performed in the control and patient (test) groups on omeprazole (20 mg/d). Billroth II gastrectomy control group did not undergo this study because of the low fraction time of gastric pH < 4 recorded during treatment-free gastric pH monitoring. Omeprazole therapy (20 mg/d) significantly reduced the 24-h fraction time of gastric pH < 4 in both control and patient groups.

Twenty-four hour gastric pH studies in patient group

Eight out of the ten patients underwent all (baseline 20 mg/day and 40 mg/day omeprazole) 24-h gastric pH studies. One additional patient underwent only baseline, and another patient both baseline and omeprazole (20 mg/day) 24-h gastric pH studies. The 24-h fraction time of gastric pH < 4 (80, 46 - 95%) was significantly reduced by omeprazole (20 mg/d) to 32, 13 - 70% (U = 6, p = 0.002) and by omeprazole (40 mg/d) to 11, 1 - 40% (omeprazole 20 mg/day vs 40 mg/day, U = 9, p = 0.01).

DISCUSSION

The data of the present study showed that recurrent ulcers after gastric surgery for duodenal ulcer can heal after 2 months of 40 mg/day omeprazole therapy. Even more important, none of our patients had a re-bleeding episode while on omeprazole maintenance therapy over a 3.5-year follow-up period.

A large review is available on the complications associated with ulcer recurrence following gastric surgery including 130 studies published on this topic over a 30-year period. The authors estimated that vagotomy plus drainage is associated with a 9% recurrence rate and a risk of hemorrhage of 1.7%. Partial gastrectomy has a lower recurrence rate (< 1%) but a similar risk of hemorrhage (1.3%). Such patients with recurrent ulcer bleeding are often submitted to a second gastric operation to cure recurrent ulcer. Similarly, most of our patients had a history of two or more hospital admissions for recurrent ulcer bleeding after surgery and two of them had a second gastric operation that failed to cure recurrent ulcer.

Cimetidine has been used in a few clinical trials including a small number of patients with recurrent ulcer bleeding following surgery for peptic ulcer disease. Cimetidine heals about 85% of ulcers within 8 weeks, but about 30% of ulcers relapse within 6 months after discontinuing therapy. Furthermore, 33% of ulcers recur 1 year after surgery while on cimetidine maintenance therapy[6], probably because of tolerance to H2RA, which is

more significant after 4 wk of therapy. Finally, there is only one clinical trial using omeprazole therapy for 12 patients with recurrent ulcer after vagotomy or gastrectomy and all ulcers are healed after 8 weeks of omeprazole therapy[8], suggesting that maintenance therapy with PPIs is the only alternative for surgical intervention. Since as many as one-third of patients with anastomotic ulcer have recurrent ulcer within a year after discontinuing acid reduction therapy, an open trial of PPI maintenance treatment should be considered ethically justifiable.

The role of H pylori in recurrent peptic ulcer disease after gastric surgery has been investigated in several clinical studies, showing that H pylori does not play an important role in post surgical ulcer recurrence. Less than half of our patients with recurrent ulcer bleeding were H pylori-positive upon admission to the study. Furthermore, one of our patients who had successful H pylori eradication therapy during a previous hospitalization was admitted to our department for a new episode of anastomotic ulcer bleeding. Another patient had two gastric operations for recurrent ulcer bleeding. Upon study entry, he had successful H pylori eradication therapy, followed by omeprazole (20 mg/d) maintenance therapy. He was healthy for 2 years and then lost his follow-up. He was readmitted to another hospital for recurrent ulcer bleeding 8 mo after discontinuing PPI maintenance therapy, though he was H pylori-negative.

With regard to the underlying mechanism of ulcer recurrence, our study showed that patients with recurrent ulcer bleeding after truncal vagotomy or partial gastrectomy had a higher treatment-free 24-h intragastric acidity than controls with no ulcer recurrence after the same operation, suggesting that vagotomy may be incomplete due to a retained antrum. Our data showed that ulcer healing therapy with omeprazole (40 mg/d) could strongly inhibit gastric acid secretion. The intragastric acidity could also be significantly reduced with oral omeprazole maintenance treatment (20 mg/d). Other factors that may be implicated in ulcer recurrence after surgical treatment for duodenal ulcer include a decreased resistance to acid in the jejunal mucosa, the site of pyloroplasty and anastomosis, possibly due to local ischemia and scarring. Hirschowitz and Lanas also showed that aspirin abusers develop intractable recurrent ulceration following gastric surgery for peptic ulcer disease. However, our patients refused to use non-steroidal anti-inflammatory drugs and their serum salicylates were negative. Zollinger-Ellison syndrome was also excluded by normal fasting serum gastrin.

In conclusion, omeprazole (20 mg/d) maintenance therapy should be the treatment of choice for patients with recurrent ulcer after gastric surgery for duodenal ulcer disease.

A novel antioxidant and antiapoptotic role of omeprazole to block gastric ulcer through scavenging of hydroxyl radical

Biswas K, Bandyopadhyay U, Chattopadhyay I, Varadaraj A, Ali E, Banerjee RK
Department of Physiology, Indian Institute of Chemical Biology, 4, Raja S. C. Mullick Road, Kolkata 700 032, India.

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 lesions, 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

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- USFDA approved in all the acid pepsin diseases and for OTC use.

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SK•F

OTC drug in the UK

Ridon
(Domperidone 10 mg tablet, 60 ml and 100 ml suspension)

...unique option in diabetic gastroparesis

- Demonstrates outstanding efficacy in symptom-relief of diabetic gastroparesis.
- Provides better outcome than metoclopramide in reducing the total symptom score.
- Unlike metoclopramide, guarantees better CNS tolerability.

Manufactured by: **ESKAYEF BANGLADESH LTD.**

