Background and Aim: Lansoprazole, a benzimidazole derivative, is a widely-used proton-pump inhibitor. In addition, it has been reported to have an independent gastroprotective action. Since free radicals and antioxidant mechanisms appear to counteract tissue-related injury, we studied the effect of lansoprazole on oxidative stress in acid-ethanol gastric injury. As this drug is metabolized in the liver, we also studied its effect on the liver. Methods: Wistar rats were divided into three groups: control group, group I (vehicle treatment) and group II (lansoprazole treatment for eight days). In all the groups, injury was induced by ethanol-HCl administration. The effect of lansoprazole on free-radical generation and various antioxidants, e.g., superoxide dismutase (SOD), catalase, reduced glutathione (GSH), glutathione-s-transferase (GST) and glutathione reductase was evaluated in the gastric mucosal and liver homogenates. Results: Ethanol-HCl administration initiated injury as shown by increase in malondialdehyde (MDA) levels in both gastric mucosa and liver. There was an increase in SOD and GST activity and a decrease in catalase, glutathione reductase and GSH in the gastric mucosa. In liver, ethanol-HCl administration decreased the activity of SOD, catalase and GSH, and increased GST activity. Lansoprazole pretreatment led to decrease in the levels of MDA and increase in SOD, catalase, GSH, glutathione reductase and GST in both the gastric mucosa and liver. Conclusions: Lansoprazole has a protective action on gastric mucosa and the liver. This protection is mediated by a decrease in oxidative stress and a concomitant increase in antioxidants. [Indian J Gastroenterol 2007;26:118-121]

Benzimidazole derivatives, such as lansoprazole, are potent proton-pump inhibitors and inhibit gastric acid secretion. It has been suggested that these drugs might also exert acid-independent mucosal protective action.1,2 However, the mechanisms involved in this protective action are unclear.

Reactive oxygen species are implicated in the pathogenesis of several diseases. Free radicals are continuously produced during normal physiologic events and removed by antioxidant defense mechanisms, including enzymes such as superoxide dismutase (SOD), catalase (CAT) and enzymes involved in the glutathione redox cycle.3

We studied the role of oxidative stress and antioxidants in lansoprazole-mediated protection in experimental mucosal injury. We hypothesized that if the protective action of lansoprazole is independent of its acid inhibitory potential, it should show similar action in other organs. The effect of lansoprazole on liver was also studied as this drug is metabolized in the liver.

Methods

Female Wistar rats weighing 150-200 g were obtained from Central Animal House, Panjab University, Chandigarh. The ethical guidelines issued by the Institute were followed for maintaining and sacrificing the animals during the course of the study. They were housed in polypropylene cages in the animal house at 22°-24°C and were fed standard pellet diet. Rats were acclimatized for seven to ten days before being used in the experimental study. They were fasted overnight before the experiment.

The animals were divided into three groups of six rats each: a) Control group was administered polyethylene glycol (PEG) orally for eight days. b) Group I animals were given PEG orally for eight days. Twelve hours after the last dose, gastric mucosal injury was induced by administering (1 mL/200 g) a mixture of 600 mL/L ethanol in 150mM HCl. c) Group II: Lansoprazole (90 µmoles/Kg) dissolved in PEG was given orally to the animals for eight days. This was followed by induction of gastric injury by ethanol-HCl twelve hours after the last dose.

Capsules of Lansol-30 were purchased from Cipla, Mumbai. All the chemicals used in the study were of analytical grade.

The animals were sacrificed by cervical dislocation; in groups I and II this was done 90 min after ethanol-HCl administration. The stomach and liver of all the animals were removed rapidly and
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washed in ice-cold normal saline. The mucosa was scraped from the stomach by sterilized blade. Liver was perfused with normal saline prior to its removal.

The mucosal scrapings and liver were weighed and minced, and gastric mucosal (100 g/L) and liver (200 g/L) homogenates were prepared in 20mM Tris-HCl buffer (pH 7.4) at 0-4°C using a motor driven Teflon pestle rotated at 3000 rpm for 10 min. The homogenate was filtered through a double layer of muslin cloth and stored for further assays. The homogenate was centrifuged at 10,000 g for 20 min at 4°C using cold centrifuge. The supernatant was termed as post-mitochondrial supernatant and used for further experiments.

Assays

Malondialdehyde (MDA) levels in the samples were determined to obtain quantitative estimation of the membrane lipid oxidative damage. MDA was assayed in terms of thiobarbituric acid reactive substrates.4 Nitrite estimation was done by the method of Green et al.5 Briefly, the homogenates were incubated with Griess reagent (10 g/L naphtyethylenediamine dihydrochloride and 10 g/L sulphanilamide in 50 mL/L phosphoric acid) at room temperature for 10 min, and the absorbance was read at 546 nm. A standard curve was prepared using sodium nitrite.

SOD activity assay was done according to the method of Kono.6 One unit of enzyme was defined as the enzyme concentration required to inhibit the reduction of nitroblue tetrazolium dye by 50% in 1 min under assay conditions, and expressed as specific activity in U/mg protein. Catalase was assayed spectrophotometrically following a decrease in absorbance of hydrogen peroxide at 240 nm, and specific activity was expressed as U/mg protein.7 Reduced glutathione (GSH) content was estimated according to the method of Ellman.8 The measurement was based on reduction with 5, 5′-dithiobis-(2-nitro-benzoic acid) and the optical density was measured at 412 nm.

Glutathione reductase was assayed by incubating PMS with NADPH and reduced glutathione.9 The change in absorbance was recorded at 340 nm for 3 min at 30-second intervals. The values were expressed as µmoles/min/mg of protein. Assay of glutathione-s-transferase (GST) activity was based on the rate of increase in conjugate forma-

tion between GSH and 1-chloro-2, 4-dinitrobenzene (CDNB).10 Enzyme activity was determined by monitoring the changes in absorbance at 340 nm for 3 min at 30-second intervals, and expressed as µmole/min/mg of protein.

The protein content in the sample was assayed by the method of Lowry et al.11

Statistical analysis

The results are given as mean (standard deviation). Statistical significance was evaluated by Student’s t test for unpaired data. p values lower than 0.05 were considered significant.

Results

MDA levels were similar in the gastric mucosa and liver (Table). There was significant increase in MDA levels on administration of ethanol-HCl. Pretreatment with lansoprazole decreased MDA level.

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Values are mean (SD). *p<0.001 as compared to control; †p<0.001 as compared to group I; ‡p<0.05 as compared to control; ‡‡p<0.05 as compared to group I; ††p<0.01 as compared to group I; †‡p<0.01 as compared to control
Nitrite levels in the gastric mucosa decreased following exposure to ethanol-HCl, while lansoprazole treatment increased the nitrite levels (Table). In the liver, there was no alteration in nitrite levels on ethanol-HCl administration. Lansoprazole treatment lowered the levels of nitrite.

Ethanol-HCl administration resulted in increase in the activity of SOD and decrease in CAT activity in gastric mucosa (Table). In the liver, there was no change in the activity of SOD and CAT with initiation of injury. Lansoprazole increased the activity of SOD and CAT in both gastric mucosa and liver.

Gastric mucosal injury by ethanol-HCl decreased the levels of GSH and glutathione reductase, while lansoprazole pretreatment increased their levels. GST activity increased on ethanol-HCl instillation. This increased further following pretreatment with lansoprazole.

In the liver, ethanol-HCl administration did not alter the levels of GSH. There was no change in the activity of glutathione reductase also, but it increased the activity of GST. Lansoprazole increased the level of GSH and the activities of glutathione reductase and GST.

**Discussion**

The results of the present study suggest that the protective effect of lansoprazole can be attributed to a decrease in oxidative stress. The protective effect was seen not only in the gastric mucosa but also in the liver, suggesting that this action is independent of its proton-pump inhibitory potential.

A mixture of ethanol-HCl has been shown earlier to produce gastric mucosal injury. Ethanol is well known to induce damage in the liver. In the present study there was an increase in MDA production following ethanol-HCl instillation in both the gastric mucosa and liver. Pretreatment with lansoprazole prevented this increase. These results are in agreement with previous reports on the gastric mucosa.

Nitrite represents a circulating and tissue storage form of nitric oxide (NO). The role of NO in gastroprotection is ambiguous. In this study, there was an increase in nitrite levels in gastric mucosa after lansoprazole treatment. This is contradictory to a previous report that had shown no effect of lansoprazole on NO in the secretory fluids. NO is an important regulator of mucus secretion in the stomach and of mucosal repair. Therefore, an increase in NO may have a protective function. Our results are supported by Hsu and coworkers who reported an increase in nitrite levels in epinephrine-mediated protection against severe acute gastric bleeding. The liver showed no change in nitrite levels following lansoprazole treatment.

Superoxide dismutase catalyzes the dismutation reaction of the toxic superoxide radicals to molecular oxygen and hydrogen peroxide. Catalase promotes the conversion of hydrogen peroxide to water and molecular oxygen. In the gastric mucosa there was an increase in SOD following ethanol-HCl instillation while catalase activity showed a decrease. Similar results have been reported in a study on peptic ulcers. Lansoprazole pretreatment increased activities of both SOD and catalase in gastric mucosa, suggesting that these enzymes may play a role in its protective action. In the liver there was an initial decrease in SOD and catalase activity following ethanol-HCl exposure. Ethanol is known to decrease SOD and catalase activity in the liver. Lansoprazole pretreatment increased the activity of both the enzymes in the liver, underlining their role in its protective action.

Sulfhydryl compounds such as GSH play an important role in scavenging reactive oxygen species and have been implicated to protect the gastric mucosa and liver. In the present study, exposure to ethanol-HCl led to a decrease in the levels of GSH in gastric mucosa as well as liver. A decrease in GSH levels in gastric mucosa after ethanol-HCl administration and in liver following ethanol treatment has been reported earlier. Lansoprazole treatment increased the GSH content in both the gastric mucosa and liver. Therefore, the antioxidant property of lansoprazole might be attributed to an increase in bioavailability of endogenous sulfhydryl groups. Other workers have also reported the role of GSH in lansoprazole-mediated gastroprotection.

Glutathione reductase is a flavoprotein that catalyzes the NADPH-dependent reduction of oxidized glutathione to reduced glutathione (GSH). GST has an important role in drug metabolism. In the present study, glutathione reductase showed a decrease while GST increased in gastric mucosa after ethanol-HCl exposure. In liver, glutathione reductase activity was not altered; this supports our findings on GSH levels, which did not show
significant change following ethanol-HCl exposure. This can be because ethanol injury was initiated for only 90 minutes, which may not be sufficient to show alteration in GSH levels and glutathione reductase activity. But an increase in the activity of glutathione reductase and GST was observed after lansoprazole treatment in both the gastric mucosa and liver. These results corroborate the findings of an increase in GSH and suggest that these are an important defense mechanism against free radicals.

In conclusion, this study suggests that the protective effects of lansoprazole could be ascribed to a decrease in oxidative stress and an increase in various antioxidants. The protective effect of lansoprazole is independent of its proton-pump inhibitory action as similar results are observed in the liver.

References

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