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Anti-ulcerogenic effect of chitin and chitosan on mucosal antioxidant defence system in HCl-ethanol-induced ulcer in rats

R. Anandan, P. G. Viswanathan Nair and Suseela Mathew

Abstract

The anti-ulcerogenic effect of chitin and chitosan against ulcer induced by HCl–ethanol in male Wistar rats was studied. Levels of acid output, pepsin, protein, lipid peroxides and reduced glutathione and the activity of glutathione peroxidase (GPx), glutathione-S-transferase (GST), catalase (CAT) and superoxide dismutase (SOD) were determined in the gastric mucosa of normal and experimental groups of rats. A significant increase in volume and acidity of the gastric juice was observed in the ulcer-induced group of rats. Peptic activity was significantly decreased as compared with that of normal controls. In the rats pre-treated with chitin and chitosan 2% along with feed, the volume and acid output and peptic activity of gastric mucosa were maintained at near normal levels. The level of lipid peroxidation was significantly higher in the ulcerated mucosa when compared with that of normal controls. This was paralleled by a decline in the level of reduced glutathione and in the activity of antioxidant enzymes like GPx, GST, CAT and SOD in the gastric mucosa of ulcer-induced rats. Also, the levels of mucosal proteins and glycoprotein components were significantly depleted in ulcerated mucosa. The pre-treatment with chitin and chitosan was found to exert a significant anti-ulcer effect by preventing all the HCl-ethanol-induced ulcerogenic effects in experimental rats.

Introduction

Peptic ulcer is a sore or hole in the lining of the stomach or duodenum (the first part of the small intestine). It occurs as a result of a disturbance of the natural balance between aggressive acid–pepsin and mucosal defence–mucosal turnover (Piper & Stiel 1986). *Helicobacter pylori* is responsible for the majority of peptic ulcers. *H. pylori* weakens the protective mucous coating of the stomach and duodenum, which allows acid to get through to the sensitive lining beneath (Blaser 1990). The last several decades have witnessed enormous progress in ulcer drug discovery and development, including a Nobel Prize to Sir James Black for his seminal work on histamine H2 receptors and their blockers, a mainstay of current drug management of ulcers. Despite these advances, the ulcer remains somewhat of a medical enigma (Glavin & Szabo 1992).

Ulcer induced by a mixture of HCl and ethanol has been reported to show many metabolic and morphologic aberrations in the gastric mucosa of experimental animals similar to those observed in human peptic ulcer (Hara & Okabe 1985). Administration of HCl–ethanol is known to produce ulcerative lesions and increase lipid peroxidation in the gastric mucosa, which plays a significant part in the pathogenesis of mucosal lesions (Pihan et al 1987). HCl–ethanol-induced oxidative damage is generally attributed to the formation of the highly reactive hydroxyl radical (OH•), stimulator of lipid peroxidation, leading to destruction of the mucosal membrane (Yoshikawa et al 1993). Alteration in the activity of antiperoxidative enzymes (superoxide dismutase (SOD) and catalase (CAT)) and glutathione-dependent antioxidant enzymes (glutathione peroxidase (GPx) and glutathione-S-transferase (GST)) has been reported in experimentally induced ulcer in rats (Morenkova et al 1987; Anandan et al 1999a).

Chitin, a polymer of *N*-acetyl glucosamine, is present in the exoskeleton of shrimp, krill, crab, squilla, insects, squid pen and fungi. Chitin is deacetylated using concentrated alkali to produce chitosan. The shrimp and cuttle-fish exoskeleton powder have

Biochemistry and Nutrition Division, Central Institute of Fisheries Technology, Matsyapuri (PO), Cochin-682029, India

R. Anandan,
P. G. Viswanathan Nair,
Suseela Mathew

Correspondence: R. Anandan, Biochemistry and Nutrition Division, Central Institute of Fisheries Technology, Cochin-682029, India.
E-mail: kranandan@email.com

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been used in indigenous medicine for the treatment of gastric ulcer. Also, Ito et al (2000) reported that repeated oral administration of chitosan accelerated the healing of gastric ulcer in experimental rats. There is little documentary evidence, however, regarding the exact mechanism involved in the anti-ulcerogenic effects of chitin and chitosan. Moreover, the effects of chitin and chitosan on gastric antioxidant status in experimentally induced ulcerated condition have not yet been explored. Hence, in this study, an attempt has been made to assess the anti-ulcer activity of chitin and chitosan on the mucosal defence system in HCl–ethanol-induced ulcer in male albino rats.

Materials and Methods

Chemicals

Adrenaline (epinephrine), tetramethoxypropane and D-galactosamine were obtained from the Sigma Chemical Co. (St Louis, MO). All other chemicals used were of analytical grade. Chitin (MW 1.08×10^5 KDa; purity 97.2%) and chitosan (MW 750 000 Da; viscosity 8 cps; deacetylation rate 85–87%; purity 98.6%) was prepared from dried prawn shell in our laboratory (Madhavan 1992).

Animals

Wistar strain male albino rats, 120–150 g, were used. They were housed under standard environmental conditions and allowed free access to food and water. Rats were deprived of food for 24 h before ulcer induction. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, and approved by the institutional Animal Ethics Committee.

Experimental protocol

The rats were divided into four groups of six and housed individually in polypropylene cages. Group I was normal control and received the standard diet for 30 days. In Group II, ulcer was induced by oral administration of 1.5 mL of 0.15 M HCl in 70% v/v ethanol (Hara & Okabe 1985) after 30 days of feeding with standard diet. Group III rats were provided with a standard diet mixed with chitin 2% for a period of 30 days and ulcer was induced as described in Group II. Group IV rats were fed with chitosan 2% in the diet for 30 days before the induction of ulcer. At the end of the experiment, all four groups underwent surgery.

The operative procedure adopted was that of Takeuchi et al (1976). The rats were anaesthetized with chloroform and the abdomen was opened through a midline incision. The pylorus was secured and ligated with silk sutures, after which the wound was closed and the rats were allowed to recover from anaesthesia. Gastric juice was then collected for a period of 4 h.

The rats were sacrificed with an overdose of chloroform and the stomach was removed after the oesophagus had been clamped. The gastric juice collected was centrifuged and the volume was noted. The stomach was inflated with saline and then examined for the number of lesions. The acid output was determined by titrating with 0.1 M NaOH using phenolphthalein as indicator.

The mucosal tissue scraped from the stomach was used for the estimation of peptic activity (Anson 1938), lipid peroxides (Ohkawa et al 1979), reduced glutathione (Ellman 1959), and the activity of CAT (Takahara et al 1960), SOD (Misra & Fridovich 1972), GPx (Pagila & Valentaine 1967) and GST (Habig et al 1974). The protein (Lowry et al 1951), hexose (Niebes 1972), hexosamine (Wagner 1979) and sialic acid (Warren 1959) contents were also determined.

Statistics

Results are expressed as mean $\pm$ s.d. One-way analysis of variance was carried out, and the statistical comparisons among the groups were performed with Tukey's test using a statistical package program (SPSS 10.0 for Windows).

Results and Discussion

The oral administration of HCl and ethanol (0.15 M HCl in 70% v/v ethanol) to rats caused development of lesions in the gastric mucosa, an increase in the volume of gastric juice and acid output, and a decrease in the activity of pepsin (Table 1). The number of lesions present on the gastric mucosa is an indication of the severity of ulcer disease (West 1982). The significant reduction observed in the number of lesions in Group III and Group IV rats might be due to the inhibition of gastric acid secretion. Acid inhibition accelerates ulcer healing (Inauen et al 1988).

An increase was noticed in acid output and volume of the gastric juice in ulcer-induced rats. The increased secretion of acid might be a consequence of increased permeability of the gastric mucosa (Dayton et al 1983). The oral administration of ethanol probably inhibited the activity of pepsin either by inactivation of pepsinogen or by affecting the conversion of pepsinogen into pepsin. Higher concentration of ethanol has already been reported to denature the enzyme pepsin (Juhani 1982). Rats pretreated with chitin and chitosan showed a significant decrease in the acid output when compared with the ulcerated group. This might be due to the acid-neutralizing capability of chitin and chitosan by the gradual release of glucosamine residues into the gastric mucosa. The peptic activity was also found to be elevated in Group III and Group IV rats, indicating the cytoprotective activity of chitin and chitosan.

Oral administration of HCl–ethanol induced a significant increase in lipid peroxidation in the gastric mucosa, which was paralleled by the reduction in the level of reduced glutathione and in the activity of antiperoxidative enzymes (SOD and CAT) and glutathione-dependent antioxidant enzymes (GPx and GST) (Table 2). Increase in the level of lipid peroxides in the ulcerated gastric mucosa reflected the damage to the mucosal cell membrane (Pihan et al 1987). Lack of antioxidant defence

Table 1 No. of lesions, volume of gastric juice, acid output and pepsin activity of the gastric mucosa of normal and experimental groups of rats.

Groups	Group I	Group II	Group III	Group IV
Number of lesions	—	9.43 ± 1.18**	2.08 ± 0.34**$^{\#}$	3.61 ± 0.48**$^{\#,\dagger}$
Volume of gastric juice (mL/4 h)	1.83 ± 0.12	2.86 ± 0.18**	2.05 ± 0.15*$^{\#}$	2.26 ± 0.14**$^{\#,\dagger}$
Acid output (μEq/4 h)	179.0 ± 15.4	302.0 ± 23.8**	213.0 ± 18.2*$^{\#}$	211.0 ± 14.2**$^{\#}$
Pepsin (μmol tyrosine/4 h)	657.0 ± 44.2	522.0 ± 30.6**	$629.0 \pm 35.7^{\#}$	594.0 ± 28.5*$^{\#,\dagger}$

Group I, normal control, rats received standard diet for 30 days; Group II, ulcer was induced by oral administration of 1.5 mL 0.15 M HCl in 70% v/v ethanol after 30 days of feeding with standard diet; Group III, rats were provided with a standard diet mixed with chitin 2% for a period of 30 days and ulcer was induced as for Group II; Group IV, rats were fed with chitosan 2% in the diet for 30 days before the induction of ulcer. Results are mean $\pm$ s.d. for 6 rats. *$P < 0.05$, **$P < 0.001$, significantly different compared with Group I control rats; #$P < 0.001$, significantly different compared with Group II ulcer-induced rats; †$P < 0.05$, significantly different compared with Group III rats.

Table 2 Levels of lipid peroxides (LPO) and GSH and the activity of GPx, GST, SOD and CAT in the gastric mucosa of normal and experimental groups of rats.

Groups	Group I	Group II	Group III	Group IV
LPO (nmol (mg protein)$^{-1}$)	3.32 ± 0.25	7.98 ± 0.57**	$3.71 \pm 0.2^{\#}$	5.18 ± 0.36*$^{\#,\dagger}$
GSH (μmol (mg protein)$^{-1}$)	4.12 ± 0.38	1.82 ± 0.22**	$3.85 \pm 0.36^{\#}$	2.90 ± 0.33*$^{\#,\dagger}$
GPX (U (mg protein)$^{-1}$)	236 ± 33	145 ± 24**	$253 \pm 31^{\#}$	194 ± 28*$^{\#,\dagger}$
GST (μmol CDNB conjugated/min/mg protein)	4.37 ± 0.27	2.62 ± 0.15**	$3.84 \pm 0.22^{\#}$	$4.07 \pm 0.18^{\#}$
SOD (U (mg protein)$^{-1}$)	5.16 ± 0.39	2.12 ± 0.25**	$4.53 \pm 0.33^{\#}$	3.25 ± 0.218*$^{\#,\dagger}$
CAT (μmol H_2O_2 consumed/min/mg protein)	3.83 ± 0.24	1.47 ± 0.11**	$3.78 \pm 0.26^{\#}$	3.16 ± 0.18*$^{\#,\dagger}$

Group I, normal control, rats received standard diet for 30 days; Group II, ulcer was induced by oral administration of 1.5 mL 0.15 M HCl in 70% v/v ethanol after 30 days of feeding with standard diet; Group III, rats were provided with a standard diet mixed with chitin 2% for a period of 30 days and ulcer was induced as for Group II; Group IV, rats were fed with chitosan 2% in the diet for 30 days before the induction of ulcer. Results are mean $\pm$ s.d. for 6 rats. *$P < 0.05$, **$P < 0.001$, significantly different compared with Group I control rats; #$P < 0.001$, significantly different compared with Group II ulcer-induced rats; †$P < 0.05$, significantly different compared with Group III rats.

might lead to an increase in the lipid peroxidation and subsequent deleterious effects. Depletion of glutathione is known to result in enhanced lipid peroxidation and excessive lipid peroxidation can cause increased glutathione consumption (Anandan et al 1999b), as observed in this study. This depletion further increases the susceptibility of gastric mucosal cells to oxygen metabolites and acid-mediated cell damage (Mutoh et al 1991). Glutathione protects the gastric cells against oxidative damage by regulating the redox status of proteins in the mucosal cell-surface membrane (Inoue et al 1987). The pre-administration of chitin and chitosan resulted in the reduction of lipid peroxidation and elevation of glutathione level. This was probably achieved by means of their antioxidant nature (Xie et al 2001) against lipid peroxidation induced by HCl–ethanol. The unpaired electron present in the hydroxyl free radicals generated by HCl–ethanol (Ito et al 1992) may have been trapped and subsequently dismuted by chitin and chitosan or by their derivatives. Xue et al (1998) have already reported the antioxidant and free-radical scavenging properties of several marine polysaccharides including chitin and chitosan.

SOD and CAT, responsible for the destruction of peroxides, have a specific role in protecting the gastric mucosa against oxidative damage (Ito et al 1992; Anandan et al 1999a). A decrease in the activity of these enzymes can lead to the formation of O^{2-} and H_2O_2, which in turn can form hydroxyl radical (OH•) and bring about a number of reactions harmful to cellular and subcellular membranes. GPx offers protection to the mucosal membrane from peroxidative damage by removing hydrogen peroxide and lipid peroxide (Inoue et al 1987; Morenkova et al 1987). There was a decrease in the activity of GPx in the ulcer-induced rats, which makes mucosal cells sensitive to oxidative damage, leading to a change in the cell composition and function. The decrease observed in the activity of GPx in the gastric mucosa of ulcer-induced rats indicated that glutathione was consumed during the reaction with oxygen and peroxide radicals. Reduction in the activity of this enzyme also leads to the accumulation of oxidants and thus the subsequent oxidation of lipid. The significant decline noticed in the activity of GST, another scavenging enzyme involved in the removal of toxic metabolites by glutathione conjugation reactions, in the gastric mucosa of ulcer-induced rats might have been due to the reduced availability of GSH. Similar observations have been reported earlier also (Morenkova et al 1987; Anandan et al 1999a). Our results

Table 3 Levels of protein, hexose, hexosamine and sialic acid in the gastric mucosa of normal and experimental groups of rats.

Groups	Group I	Group II	Group III	Group IV
Protein (mg g^{-1})	21.50 ± 1.80	10.20 ± 1.17**	$18.70 \pm 1.58^{\#}$	$15.90 \pm 1.32^{*,\#,\dagger}$
Hexose (mg g^{-1})	14.20 ± 1.04	7.78 ± 0.54**	$12.80 \pm 0.98^{\#}$	$11.50 \pm 0.95^{*,\#}$
Hexosamine (mg g^{-1})	9.48 ± 0.72	4.26 ± 0.31**	$7.92 \pm 0.65^{*,\#}$	$7.45 \pm 0.57^{*,\#}$
Sialic acid (mg g^{-1})	1.52 ± 0.11	0.57 ± 0.08**	$1.38 \pm 0.10^{\#}$	$1.31 \pm 0.12^{\#}$

Group I, normal control, rats received standard diet for 30 days; Group II, ulcer was induced by oral administration of 1.5 mL 0.15 M HCl in 70% v/v ethanol after 30 days of feeding with standard diet; Group III, rats were provided with a standard diet mixed with chitin 2% for a period of 30 days and ulcer was induced as for Group II; Group IV, rats were fed with chitosan 2% in the diet for 30 days before the induction of ulcer. Results are mean ± s.d. for 6 rats. $^{*}P < 0.05$, $^{**}P < 0.001$, significantly different compared with Group I control rats; $\#P < 0.001$, significantly different compared with Group II ulcer-induced rats; $\dagger P < 0.05$, significantly different compared with Group III rats.

lead to the conclusion that GSH and GSH-dependent enzymes are directly related to the pathogenic mechanism of peptic ulcer.

Gastric mucosa pre-exposed to chitin and chitosan showed a decrease in lipid peroxidation, an important cause of destruction and damage to mucosal membranes (Pihan et al 1987; Yoshikawa et al 1993) and an increase in the activity of antiperoxidative enzymes and glutathione-dependent antioxidant enzymes. These findings indicate that chitin and chitosan are anti-ulcerogenic agents.

The levels of protein and glycoprotein components were significantly less in the gastric mucosa of ulcer-induced rats than in that of controls (Table 3). The mucosal proteins are easily susceptible to oxidative free radicals produced by HCl–ethanol, resulting in corrosion, disruption and disintegration of mucosal cells (Davenport 1967; Guth et al 1984). Alcohol intake is reported to increase the loss of mucosal proteins into the gastric juice in the presence of HCl (Brossinne 1979). Increased loss of protein in the ulcerated condition might be a major factor involved in the induction of ulcerative lesions of the gastrointestinal tract. The administration of HCl–ethanol might affect the glycoprotein secretion of epithelial cells by eroding the cells, leading to the decreased levels of protein and glycoprotein components.

Pre-treatment with chitin and chitosan significantly prevented the depletion of protein and glycoprotein components in the gastric mucosa as compared with ulcer-induced rats. They probably did so by strengthening the mucosal barrier, which is the first-line defence against exogenous and endogenous ulcerogenic agents. Hence, chitin and chitosan can be categorized as cytoprotective agents.

Conclusion

The results of this study indicate that chitin is more effective than chitosan in preventing HCl–ethanol induced gastric ulcer. The overall anti-ulcerogenic effects of chitin and chitosan are probably related to a counteraction of free radicals through their antioxidant property, or to a neutralization of gastric juice by the gradual release of glucosamine or to their ability to maintain near to normal status the activity of free-radical enzymes and the level of GSH, which protects mucosa against oxidative damage by decreasing lipid peroxidation and strengthening the mucosal barrier.

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