Biochemical Studies on the Antiulcer Effect of Glucosamine on Antioxidant Defense Status in Experimentally Induced Peptic Ulcer in Rats

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Summary **The present study examined the antiulcer effect of glucosamine on mucosal antioxidant defense system in ibuprofen-induced peptic ulcer in male albino rats. The number of lesions in the gastric mucosa, volume of gastric juice, acid output, pepsin activity, lipid peroxides, reduced glutathione content and the activities of glutathione dependent antioxidant enzymes (glutathione peroxidase and glutathione-*S*-transferase) and antiperoxidative enzymes (catalase and superoxide dismutase) were determined. Prior oral administration of glucosamine significantly prevented the ibuprofen-induced increases in the number of lesions in the gastric mucosa, volume of gastric juice and acidity. It also maintained the activity of pepsin at near normal level. Oral pretreatment of glucosamine exerted a significant antioxidant effect by preventing ibuprofen-induced lipid peroxidation and by maintaining the level of reduced glutathione and the activities of mucosal antioxidant enzymes at near normalcy. The results of the present investigation indicate that the antiulcer activity of glucosamine is related to its ability to neutralize the hydrochloric acid secreted into the stomach and to its antioxidant capability to inhibit ibuprofen-induced lipid peroxidation.**

Key Words: **glucosamine, ibuprofen, ulcer, acid out put, antioxidant status**

Introduction

Peptic ulcer is a non-malignant ulcer of the stomach or duodenum. A major causative factor (90% of gastric and 75% of duodenal ulcers) is chronic inflammation due to *Helicobacter pylori*, a spiral-shaped bacterium that lives in the acid environment of the stomach [*1*]. These ulcers can also be caused or worsened by drugs such as ibuprofen and other NSAIDs [*2*]. The lesion of peptic ulcer is a disruption in the mucosal layer of the stomach or duodenum [*3*]. Peptic ulcer represents the imbalance between defensive factors that protect the mucosa and offensive or aggressive factors that disrupt this important barrier [*4*]. Some mucosal protective factors include the water-insoluble mucous gel layer, local production of bicarbonate, regulation of gastric acid secretion, and adequate mucosal blood flow [*5*]. Aggressive factors include the acid-pepsin environment, infection with *H. pylori*, and mucosal ischemia [*6*].

Ulcer induced by ibuprofen shows many metabolic and morphologic aberrations in the gastric mucosa of experimental animals similar to those observed in human peptic ulcer [*7*]. It is induced by a multiple

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step mechanism. In particular, the peroxidation of mucosal endogenous lipids has been shown to be a major factor in the cytotoxic action of ibuprofen [*8*]. Ibuprofen-induced oxidative damage is generally attributed to the formation of the highly reactive hydroxyl radical ($^{\bullet}OH$), stimulator of lipid peroxidation and source for destruction and damage to the mucosal cell membrane [*9*]. Alterations in the level of reduced glutathione (GSH) and the activities of glutathione-dependent antioxidantenzymes [glutathione peroxidase (GPX) and glutathione-*S*-transferase (GST)] and antiperoxidative enzymes [catalase (CAT) and superoxide dismutase (SOD)] have been reported in experimentally induced peptic ulcer condition in rats [*10*].

Traditional medicines have been the starting point for the discovery of many important modern drugs. This fact has led to the chemical and pharmacological investigations and general biological screening programs for natural products all over the world. Prawn shell, a main waste material of seafood industry, can be efficiently utilized by converting it into various useful by-products like chitin, chitosan, glucosamine *etc.* [*11*]. In traditional medicine, the shrimp and cuttlefish exoskeleton powder has been used to cure arthritis, diabetes, stomach disorders, epilepsy and various liver disorders. Earlier report [*12*] from our laboratory indicated that the supplementation of chitin and chitosan, along with feed, prevented HCl-ethanol mixture induced peptic ulcer in experimental rats. Glucosamine is the basic unit of chitin and chitosan and it is an essential component required for glycoprotein synthesis in living beings [*13*]. The beneficial actions of chitin and chitosan may be ascribable to their basic unit glucosamine. Hence, it is thought to be important to study the preventive effect of glucosamine on experimentally induced ulcer condition to derive a conclusion regarding the exact biochemical mechanism involved in the protective effects of shrimp and cuttlefish exoskeleton powder in alleviating the ulcerative disorders. In the present study, we assessed the antiulcer effects of glucosamine on mucosal antioxidant system in ibuprofen-induced ulcer in rats.

Materials and Methods

Drugs and chemicals

Ibuprofen was obtained from Cipla pharmaceuticals, Mumbai, India. Bovine serum albumin, tetraethoxypropane, epinephrine and D-galactosamine were obtained from M/s Sigma Chemical Company, St. Louis, MO, USA. All other chemicals used were of analytical grade.

Animals

Male Wister rats (weighing 120–150 g) were allowed to undergo standard pelletted diet and water and housed under standard environmental conditions. Animals were deprived of food for 24 h prior to ulcer induction.

Experimental protocol

The experimental animals were divided into four groups of six rats each. Rats in Group I (normal control) received only the standard diet. In Group II, normal rats were treated with glucosamine (100 mg/kg/day, p.o for 20 days). In Group III, ulcer was induced by oral administration of ibuprofen (50 mg/kg, p.o) twice in a day at an interval of 12 h. Group IV was pretreated with glucosamine (100 mg/kg/day, p.o for 20 days) before induction of ulcer as described for Group III. At the end of the experiment, all four groups underwent surgery according to Takeuchi *et al.* [*14*] and the gastric juice was collected for 4 h. Rats were then killed with over dose of chloroform and the stomach was removed after the esophagus had been clamped. The gastric juice was centrifuged and the volume was noted. The total acidity was determined by titrating with 0.02 N NaOH with phenolphthalein used as indicator. The mucosal tissue scrapped was used for the determination of pepsin [*15*], lipid peroxides (LPO) [*16*], GSH [*17*], GPX [*18*], GST [*19*], CAT [*20*] and SOD [*21*].

The experiment was carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India and approved by the Institutional Animal Ethics Committee (IAEC).

Statistical analysis

The values were expressed as mean±SD for six animals. The statistical significance was performed by using one way ANOVA followed by Tukey's test using SPSS10 Windows Software.

Results

Table 1 shows the number of lesions present in the gastric mucosa, volume of gastric juice, acid output and peptic activity in normal and experimental

Table 1. Number of lesions, volume of gastric juice, acid output and pepsin activity of the gastric mucosa of normal and experimental groups of rats.

	Group I Control	Group II Glucosamine (A)	Group III Ulcer (B)	Group IV (A+B)
Number of lesions	—	—	$8.78\pm1.42^{a,b}$	$1.25^{a,b,c}$
Volume of gastric juice (ml/4 h)	1.45 ± 0.05	1.37 ± 0.04	$2.76\pm0.09^{a,b}$	1.55 ± 0.05^{c}
Acid output (μEq/4 h)	156 ± 12.3	138 ± 14.7	$293\pm18.2^{a,b}$	172 ± 15.5^{c}
Pepsin (μmol tyrosine liberated/4 h)	723 ± 58.1	698 ± 63.5	$576\pm41.9^{a,b}$	682 ± 54.7^{c}

Mean±SD for six animals in each group. [a]$p<0.001$ significantly different compared with Group I control animals; [b]$p<0.001$ significantly different compared with Group II glucosamine administered rats; [c]$p<0.001$ significantly different compared with Group III ulcer induced animals.

Table 2. Levels of lipid peroxides and reduced glutathione and the activities of glutathione peroxidase, glutathione-*S*-transferase, catalase and superoxide dismutase in the gastric mucosa of normal and experimental groups of rats.

	Group I Control	Group II Glucosamine (A)	Group III Ulcer (B)	Group IV (A+B)
LPO	1.02 ± 0.02	0.96 ± 0.01	$2.45\pm0.05^{a,b}$	1.18 ± 0.01^{c}
GSH	5.02 ± 0.36	4.89 ± 0.25	$2.12\pm0.12^{a,b}$	4.73 ± 0.18^{c}
GPX	179 ± 14.2	195 ± 16.8	$108\pm7.3^{a,b}$	172 ± 15.5^{c}
GST	5.46 ± 0.38	5.34 ± 0.33	$3.18\pm0.25^{a,b}$	4.95 ± 0.34^{c}
CAT	4.08 ± 0.21	3.95 ± 0.26	$1.25\pm0.09^{a,b}$	3.82 ± 0.18^{c}
SOD	5.87 ± 0.27	6.02 ± 0.32	$2.25\pm0.18^{a,b}$	5.18 ± 0.22^{c}

Mean±SD for six animals in each group. Values are expressed as: LPO, nmol/mg protein; GSH, nmol/g wet tissue; GPX, nmol GSH oxidized/min/mg protein; GST, μmol 1-chloro-2,4 dinitrobenzene conjugate formed/min/mg protein; CAT, μmol of H_2O_2 consumed/min/mg protein; SOD, one unit of the superoxide dismutase activity is the amount of protein required to give 50% inhibition of epinephrine autoxidation. [a]$p<0.001$ significantly different compared with Group I control animals; [b]$p<0.001$ significantly different compared with Group II glucosamine administered rats; [c]$p<0.001$ significantly different compared with Group III ulcer induced animals.

groups of rats. Oral administration of ibuprofen caused significant ($p<0.001$) increase in the number of lesions in the gastric mucosa of Group III rats as compared with that of Group I control animals. Significant ($p<0.001$) rise in the volume of gastric juice and acid output was observed in ulcer-induced animals as compared with Group I rats. Also a significant ($p<0.001$) reduction in the peptic activity was noted. Oral pretreatment with glucosamine significantly ($p<0.001$) reduced the number of ibuprofen-induced lesions and maintained the levels of gastric juice volume, acid output and peptic activity at near normal level.

Table 2 shows the levels of LPO and GSH and the activities of GPX, GST, CAT and SOD in the gastric mucosa of normal and experimental groups of rats. Significant ($p<0.001$) increase in the level of lipid peroxides was noticed in the gastric mucosa of Group III ulcer induced rats as compared with Group I animals. This was paralleled by the significant decline in the level of GSH and the activities of GPX, GST, CAT and SOD. Prior oral administration of glucosamine significantly ($p<0.001$) prevented all this ibuprofen-induced adverse effects and maintained the rats at near normal status.

Discussion

Significant ($p<0.001$) increase in the number of lesions present on the gastric mucosa is indicative of the severity of ibuprofen-induced ulcer (Table 1). Increased acid secretion into the stomach is the major causative factor in the development of human peptic ulcer disease [*22*]. The significant ($p<0.001$) rise noted in the volume of gastric juice and acidity with a concomitant decline in peptic activity in the Group III rats may be due to a consequence of increased permeability of the ulcerated gastric mucosa. This concurs with an earlier reported study [*23*], which indicated that the acid suppression therapy using proton pump inhibitors or histamine H_2-receptor antagonists is an effective newer approach for reducing the risk of occurrence of peptic ulcer.

In the present study, the oral pre-treatment with

glucosamine resulted in the significant ($p<0.001$) reduction in number of lesions in the gastric mucosa, volume of gastric juice and acid output in Group IV animals as compared with Group III ulcer induced rats. Also the peptic activity was maintained at a level comparable to that of control animals. It probably did so by neutralizing the excessively secreted hydrochloric acid in the stomach. Mutoh *et al.* [*24*] have postulated that the adaptive cytoprotection in cultured gastric mucus producing cells is mediated by glucosamine rich mucus released in response to a mild irritant.

Lipid peroxidation reaction, a type of oxidative degeneration of polyunsaturated fatty acids, has been linked with altered membrane structure and enzyme inactivation. The level of lipid peroxidation was found to be significantly ($p<0.001$) higher in the gastric mucosa of Group III ulcer-induced rats as compared with that of normal controls (Table 2). Significant increases in the levels of LPO in the ulcer-induced gastric mucosa have already been reported [*25*, *26*]. Lipid peroxidation worsens the mucosal injury. The exact sources of peroxyl radicals in peptic ulcer are not yet thoroughly understood. Xanthine oxidase has been considered as one of the major source of free radicals [*27*]. Another source may be neutrophils, since administration of non-steroidal anti-inflammatory drugs has already been reported to cause neutrophil infiltration into the gastric mucosa [*28*]. Jimenez *et al.* [*29*] proposed that the active oxygen species, derived both from xanthine oxidase and activated neutrophils, could play a role in the pathogenesis of gastric injury induced by ibuprofen. The metabolism of arachidonic acid *via* the lipoxygenase and cyclooxygenase pathways may also result in the formation of reactive oxygen species and other free radicals in the ulcerated gastric mucosa [*30*].

Prior administration of glucosamine resulted in a significant ($p<0.001$) reduction in the level of LPO towards near normalcy as compared with Group III ulcer-induced rats, establishing its cytoprotective effect. The unpaired electron present in the ibuprofen-generated free radicals might have been trapped and subsequently dismuted by glucosamine by its antioxidant nature [*31*]. Reports by Chen *et al.* [*32*] showed that the dimeric form of glucosamine exerted free radical scavenging activity in a non-enzymatic system comprising of phenazine methosulfate and NADH.

GSH has a direct antioxidant function. It functions by reaction with superoxide radicals, peroxyl radicals and singlet oxygen, followed by the formation of oxidized glutathione and other disulphides [*33*]. Depletion of GSH results in enhanced lipid peroxidation, and excessive lipid peroxidation can cause increased GSH consumption [*11*], as observed in the present study (Table 2). This is in accordance with a previously reported study [*34*], which indicated that the depletion of gastric GSH by diethyl maleate produced gastric ulceration. This reduction might have resulted from the oxidation of GSH to ibuprofen-induced generation of free radicals. The depletion of GSH further enhances the susceptibility of the gastric mucosal cells to oxygen metabolites and acid mediated cell damage. Hiraishi *et al.* [*35*] has reported that exogenous GSH protects cultured gastric mucosal cells from oxidant-induced damage.

In the present study, the oral pretreatment with glucosamine maintained the level of GSH, which protects against oxidative damage by the way of regulating the redox status of proteins in the cell membrane [*11*], at near normalcy as compared with that of Group III ulcer-induced rats. This indicates that normal maintenance of mucosal GSH levels by glucosamine in ulcer rats may be the result, but not cause, of the cytoprotection by glucosamine.

Activities of glutathione-dependent antioxidant enzymes (GPX and GST) and antiperoxidative enzymes (CAT and SOD) were significantly ($p<0.001$) lower in the gastric mucosa of Group III ulcer-induced rats as compared with that of Group I control animals (Table 2). GPX, an antioxidant enzyme, offers protection to the mucosal membrane from peroxidative damage [*5*]. A significant decrease in the activity of GPX makes mucosal membrane susceptible to ibuprofen-induced peroxidative damage, which leads to changes in the gastric mucosal composition and function. GST, another scavenging enzyme, binds to many different lipophilic compounds [*33*]; so it would be expected to bind ibuprofen and act for GSH conjugation reactions. The significant ($p<0.001$) decrease in GST activity noted in this study might have been due to the decreased availability of GSH.

Reduction noticed in the activities of CAT and SOD in experimentally induced ulcerated mucosa is in line with earlier reported studies [*5*, *36*], indicating that decline in the activities of these antiperoxidative enzymes leads to the formation of peroxyl radicals, which are harmful to the gastric mucosa. In the present study, the prior oral administration of

glucosamine significantly ($p<0.001$) prevented the ibuprofen-induced alterations in the activities of glutathione-dependent antioxidant enzymes (GPX and GST) and antiperoxidative enzymes (SOD and CAT) in the gastric mucosa of Group IV rats. It probably did so by the counteraction of ibuprofen-induced free radicals [*37*] due to its antioxidant nature.

The results of the present study indicate that the overall antiulcerogenic effect of glucosamine is probably related to its ability to neutralize the excess acid secreted into the stomach and to maintain near to the normal status the activities of the mucosal antioxidant enzymes and the level of GSH, which protect mucosa against oxidative damage by decreasing the lipid peroxidation and strengthening the mucosal barrier, and which are the first line of defense against exogenous and endogenous ulcerogenic agents.

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