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Protective effect of (+)-catechin against gastric mucosal injury induced by ischaemia–reperfusion in rats

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Abstract

Ischaemia and reperfusion are known to induce gastric lesions, predominantly due to excessive formation of reactive oxygen metabolites, adhesion of neutrophils to endothelial cells, microvascular dysfunction, gastric acid secretion, endogenous histamine and gastrin release. We have studied the effect of (+)-catechin on a gastric ulcer model involving damage to gastric injury by ischaemia–reperfusion (I/R) in rats. (+)-Catechin 50 mg kg^{-1} administered orally, once daily for three days after the initiation of I/R injury showed a significant ($P < 0.001$) anti-ulcer activity against mucosal damage. However, (+)-catechin significantly decreased the lipid peroxidation and increased the level of catalase in the I/R condition. Elevated levels of alkaline phosphatase in the I/R group was significantly lowered ($P < 0.01$) by (+)-catechin. The amount of H^+K^+ATPase was significantly decreased ($P < 0.001$) in (+)-catechin-treated as compared with I/R rats. (+)-Catechin significantly decreased elevated plasma histamine ($P < 0.05$) and corticosterone ($P < 0.05$). The results suggested that (+)-catechin protected gastric mucosa against ischaemia–reperfusion-induced gastric ulcers by its antioxidant activity and mucus protection.

Introduction

Gastric ulceration is a highly prevalent disease in man that arises as a complication following burns, sepsis, major surgery, ischaemia, trauma and other heterogeneous forms of stress. Ischaemia and reperfusion are known to induce gastric lesions predominantly due to the excessive formation of reactive oxygen metabolites, adhesion of neutrophils to endothelial cells, microvascular dysfunction, gastric acid secretion, endogenous histamine and gastrin release (Brzozowski et al 2000). Ischaemia weakens the gastric mucosal barrier and increases the acid back-diffusion predisposing the gastric mucosa to damage (Kawai et al 1994). After reperfusion, the reactive oxygen species (ROS) are generated from the xanthine–xanthine oxidase system and activated neutrophils, leading to tissue lipid peroxidation, which in combination with gastric acid secretion result in cellular death and damage (Smith et al 1996; Wada et al 1996). ROS such as superoxide radical and hydroxyl radical are known to directly or indirectly cause tissue damage. They are also involved in the pathogenesis of gastric lesions observed after ischaemia–reperfusion and stress (Iino et al 2002). Indeed, several studies have shown that superoxide dismutase (SOD) and catalase protected against oxidative stress induced by ischaemia–reperfusion (Yoshikawa et al 1992; Iino et al 2002). Furthermore, it has been suggested that histamine and H^+K^+ATPase contributed to stimulate acid secretion and damage the mucosal membrane (Soumarmon et al 1983; Kitano et al 2005). Alkaline phosphatase is an enzyme capable of catalysing the hydrolysis of various phosphate esters at alkaline pH. The release of this enzyme has been suggested to play a role in tissue necrosis associated with various models of gastrointestinal ulceration (Obi et al 2000).

Flavonoids are low molecular weight polyphenolic compounds that are widely distributed in plants. In recent years, flavonoids have been more progressively used as a natural ingredient in beneficial health products. (+)-Catechin, a monomeric flavonol from the group of catechin, is known to be present in green tea, black tea and other plant foods. Recent literature suggests that catechins have beneficial effects on human health, serving to protect against cardiac failure and cancer due to their antioxidant properties (Cook & Samman 1996; Chander et al 2005; Yilmaz 2006). Tea catechins consist of epicatechin,

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epigallocatechin gallate, and epigallocatechin which protects the gastric mucosal injury against ethanol, by its antioxidant properties (Hamaishi et al 2006). However, the influence of (+)-catechin on the gastric ulcerogenic response induced by ischaemia–reperfusion (I/R) has not yet been examined. In this study, the effect of (+)-catechin on gastric ulcers induced by reperfusion in the ischaemic rat stomach, in comparison with reduced glutathione and omeprazole, has been investigated. We studied also the effect of catechin on levels of lipid peroxidation inhibition, catalase, superoxide dismutase, alkaline phosphatase, H^+K^+ATPase, histamine and corticosterone in rats with I/R-induced ulcers.

Materials and Methods

Animals

Male Sprague–Dawley rats (150–175 g) were obtained from the Central Drug Research Institute, Lucknow, and housed three to a cage for the duration of the study. Rats had free access to standard rodent pellet diet (Amrut, India) and were maintained in a temperature- and humidity-controlled environment on a 12-h dark/light cycle. Animals were fasted for 24 h before the study but water was freely available. All studies were performed in accordance with the guide for the care and use of laboratory animals, as adopted and promulgated by the Animal Care committee, CPCSEA, India (Reg. No. 222/2000/CPCSEA).

Gastric ischaemia–reperfusion and treatment

Ischaemia–reperfusion was produced in 24-h fasted rats under pentobarbital sodium anaesthesia (50 mg kg^{-1}, i.p.), a laparotomy was performed. The celiac artery was occluded with a micro-bulldog clamp for 0.5 h. At the end of the ischaemic period, the clamp was released and three drops of lidocaine were applied directly on the celiac artery to facilitate reperfusion for 60 min (Wada et al 1996). Immediately after reperfusion the surgical wound was sutured with the treatment of protecting suppuration. Rats received omeprazole (30 mg kg^{-1}, i.p.) or reduced glutathione (150 mg kg^{-1}, i.p) or (+)-catechin (50 mg kg^{-1}, p.o.) at 24, 48 and 72 h after the I/R and control animals received physiological saline (0.9% NaCl in double distilled water). The rats were killed by exsanguination via the abdominal aorta 1 h after the administration of the last dose, and the stomach was removed. The effects of all drugs were evaluated as total area of ulcers (mm^2) at 72 h after I/R as described by Rao et al (2004). The fundic part of the I/R-induced ulcer stomach was homogenized (5%) in ice-cold 0.9% NaCl with a glass homogenizer for 30 s. The homogenate was centrifuged at 800 *g* for 10 min and the supernatant was again centrifuged at 12 000 *g* for 15 min. The mitochondrial fraction obtained was used for the following estimations.

Estimation of lipid peroxidation (LPO)

A volume of the homogenate (0.20 mL) was transferred to a vial and mixed with 0.2 mL 8.1% sodium dodecyl sulfate solution, 1.5 mL 20% acetic acid solution (adjusted to pH 3.5 with NaOH) and 1.5 mL 0.8% solution of thiobarbituric acid (TBA). The final volume was adjusted to 4.0 mL with distilled water. Each vial was tightly capped and heated in a boiling water bath for 60 min. The vials were then cooled under running water. Equal volumes of tissue blank or test samples and 10% trichloroacetic acid were transferred into a centrifuge tube and centrifuged at 1000 *g* for 10 min. The absorbance of the supernatant fraction was measured at 532 nm. A control experiment was processed using the same experimental procedure except that the TBA solution was replaced with distilled water (Jamall & Smith 1985). 1,1,3,3-Tetraethoxypropan was used as the standard for calibration of the curve and results were expressed as nmol MDA (mg protein)$^{-1}$.

Catalase activity

Catalase (CAT) activity was assayed by the method of Claiborne (1985). Briefly, the assay mixture consisted of 1.95 mL phosphate buffer (0.05 M, pH 7.0), 1.0 mL hydrogen peroxide (0.019 M) and 0.05 mL homogenate in a final volume of 3.0 mL. Changes in absorbance were recorded at 240 nm. One unit (U) catalase was defined as the amount of enzyme required to decompose 1 μmol H_2O_2 min^{-1}, at 25°C and pH 7.0. Results were expressed as U CAT (mg protein)$^{-1}$.

Superoxide dismutase (SOD) activity

The assay consisted of EDTA 0.1 mM, sodium carbonate 50 mM and nitro blue tetrazolium 96 mM: the inhibition of nicotinamide adenine dinucleotide (reduced)-phenazine methosulphate-nitro blue tetrazolium reaction system (Nishikimi et al 1972; Kakkar et al 1984). One unit of the enzyme was equivalent to 50% inhibition in formazan formation in 1 min at room temperature (25°C ± 2) and the results were expressed as U SOD (mg protein)$^{-1}$.

Measurement of alkaline phosphatase activity

The gastric mucosal specimen of each rat was scrubbed off, weighed and dissolved in 2.0 mL saline at 4°C. After sonication, the homogenates were centrifuged at 3000 *g* for 10 min to separate particulate from the soluble fractions. Alkaline phosphatase was estimated by using a biochemistry kit (Merck Ltd, India). Protein concentrations were determined using the Lowry method (Lowry et al 1951), with alkaline phosphatase activity expressed in international units (IU) (mg protein)$^{-1}$.

Estimation of H^+K^+ATPase activity

The H^+K^+ATPase activity was assayed in medium consisting of 70 mM Tris buffer, pH 6.8, 5 mM $MgCl_2$ and the enzyme solution in the presence of 10 mM KCl in a total volume of 1 mL, and incubated for 1 h. The reaction was initiated by adding 2 mM ATP and further incubated at 37°C for 20 min. The reaction was stopped by 10% TCA and after centrifugation, 2.5 mL ammonium molybdate and 0.5 mL l-amino-2-naphthol-4-sulfonic acid were added to the supernatant and the absorbance was read at 620 nm (Nagaya et al 1987).

Estimation of histamine content

Histamine content was estimated in plasma by an HPLC method. The animals were lightly anaesthetized with ether and blood was collected from the supraorbital plexus using the microcapillary technique and plasma was separated. The plasma was treated with 0.2 M perchloric acid and centrifuged at 10 000 *g* for 30 min at 4°C. The clear supernatant was then used for the determination of histamine content by HPLC (Tsuruta et al 1981; Oishi et al 1987) and expressed as IU (mg protein)$^{-1}$.

Estimation of plasma corticosterone

Plasma corticosterone was estimated by the method of Glick et al (1964). The mixture contained 0.3 mL isooctane and 0.1 mL plasma. The contents were centrifuged and the isooctane was discarded, 0.6 mL chloroform was added to each tube and after extraction of 0.4 mL chloroform was transferred to another stoppered tube. To this 0.8 mL acid-alcohol (50%) solution (2:1) was added. After 1 h, acid layer fluorescence was measured at 462 nm (excitation) and (emission) using a spectrofluorimeter and results were expressed as μg dL^{-1}.

Statistical analysis

Values are expressed as means ± s.e.m. for six rats in a group. All the data were analysed by one-way analysis of variance followed by Newman–Keuls test using GraphPad Prism version 3.01. $P < 0.05$ was considered significant.

Results

Gastric ischaemia–reperfusion (I/R) resulted in damage to the muscularis mucosae and aggravated the formation of ulcers. The ulcer area was 58.6 ± 7.2 mm^2 in I/R-induced rats. Administration of (+)-catechin after the initiation of injury showed the mean value of ulcers was significantly inhibited (15.6 ± 2.7 mm^2 ($P < 0.001$)) compared with the untreated I/R group (Figure 1). Mucosal level of alkaline phosphatase in the I/R group was significantly decreased (0.10 ± 0.02; $P < 0.01$) as compared with control (0.21 ± 0.03). However, (+)-catechin enhanced the level of alkaline phosphatase to 0.18 ± 0.03 IU (Figure 1). Reduced glutathione and the proton-pump blocker omeprazole resulted in a significant reduction in ulcer area to 20.8 ± 3.9 and 12.3 ± 2.1 mm^2, respectively. Animals subjected to gastric I/R showed elevation in LPO and decrease in catalase and SOD. Treatment with (+)-catechin (50 mg kg^{-1}) significantly reduced LPO from 0.48 ± 0.02 to 0.30 ± 0.01 nmol MDA (mg protein)$^{-1}$ and increased the level of catalase from 15.5 ± 1.3 U CAT activity (mg protein)$^{-1}$ in the I/R group to 32.4 ± 1.8 U CAT activity (mg protein)$^{-1}$. SOD also increased from 87.6 ± 12.4 to 145.0 ± 7.5 U (mg protein)$^{-1}$ ($P < 0.001$). Reduced glutathione and omeprazole showed significant inhibition in LPO (0.32 ± 0.01 and 0.35 ± 0.01 nmol MDA (mg protein)$^{-1}$) and enhanced the activity of catalase (31.9 ± 1.7 and 31.5 ± 1.5 U CAT (mg protein)$^{-1}$, respectively) and SOD (149.1 ± 8.7 and 137.0 ± 7 U SOD (mg protein)$^{-1}$, respectively) activity (Figure 2).

The level of H^+K^+ATPase was significantly decreased from 1.15 ± 0.05 ($P < 0.001$) to 0.51 ± 0.03 mmol inorganic phosphate (Pi) liberated min^{-1} (mg protein)$^{-1}$ in (+)-catechin-treated as compared with I/R rats (Figure 3). On other hand,

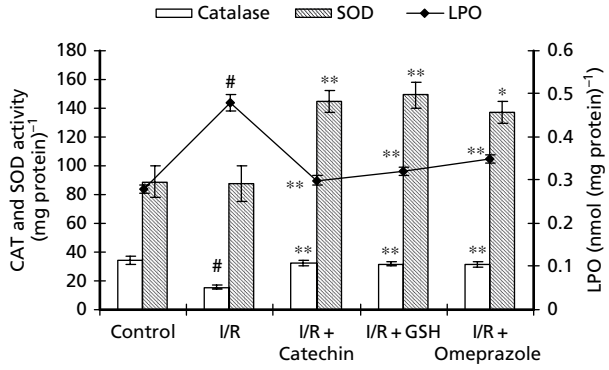

Figure 2 Effect of (+)-catechin, reduced glutathione (GSH) and omeprazole on I/R-induced lipid peroxidation (LPO), catalase and superoxide dismutase (SOD) after 72-h I/R. Values are expressed as mean ± s.e.m. *$P < 0.01$, **$P < 0.001$ compared with I/R group. #$P < 0.001$ compared with control group.

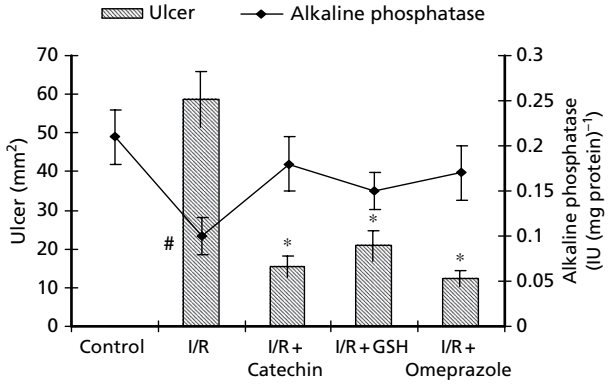

Figure 1 Effect of (+)-catechin, reduced glutathione (GSH) and omeprazole on I/R-induced ulcer (mm^2) and alkaline phosphatase after 72-h I/R. Values are expressed as mean ± s.e.m. *$P < 0.001$ compared with I/R group. #$P < 0.001$ compared with control group.

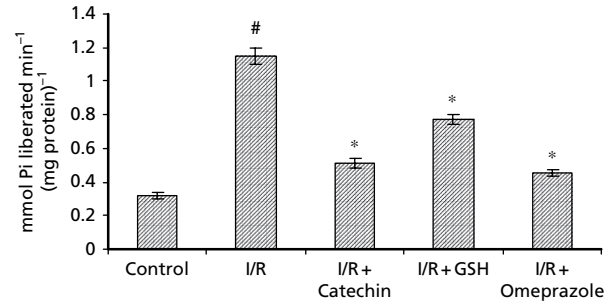

Figure 3 Effect of (+)-catechin, reduced glutathione (GSH) and omeprazole on I/R-induced H^+K^+ATPase after 72-h I/R. Values are expressed as mean ± s.e.m. *$P < 0.001$ compared with I/R group. #$P < 0.001$ compared with control group.

plasma histamine content was significantly increased in I/R-induced animals from 228 ± 23.2 to 403 ± 38.5 IU (mg protein)$^{-1}$ ($P < 0.01$), whereas (+)-catechin significantly decreased the elevated plasma histamine to 286 ± 20.7 IU (mg protein)$^{-1}$ ($P < 0.05$) (Figure 4). The mean corticosterone values play an important role in gastric ulceration and demonstrated that I/R increased significantly the level to 39.6 ± 4.0 μg dL^{-1} from the control value of 21.5 ± 3.2 ($P < 0.001$). The elevated corticosterone values in the I/R groups were significantly lowered by (+)-catechin (24.3 ± 3.7 μg dL^{-1}, $P < 0.05$), reduced glutathione (23.5 ± 3.2 μg dL^{-1}, $P < 0.05$) or omeprazole (25.2 ± 3.4 μg dL^{-1}, $P < 0.01$), respectively (Figure 4).

Discussion

The major clinical disorders involving gastrointestinal circulation are haemorrhage and ischaemia. In this study, (+)-catechin significantly decreased the total area of ulcers examined 72 h after the onset of ischaemia–reperfusion in rats. We confirmed the original observation that the exposure of the gastric mucosa to ischaemia by clamping of the celiac artery followed by reperfusion did produce deeper chronic gastric lesions and damage to the muscularis mucosa (Wada et al 1996). It is generally accepted that reactive oxygen species mediated peroxidation of lipid structures in the tissues results in extensive subcellular damage and play a major role in the pathogenesis of ischaemia–reperfusion-induced ulcers (Iino et al 2002). The increase in the thiobarbituric acid reactive substances value, the index of tissue lipid peroxidation, in the gastric mucosa after ischaemia–reperfusion was significantly inhibited by treatment with (+)-catechin. Lipid peroxidation plays an important role in the formation of gastric injury induced by reperfusion (Yoshikawa et al 1992). Superoxide and hydroxyl radicals are major reactive oxygen radicals contributing to ischaemia–reperfusion injury in the stomach. These reactive oxygen species attack and damage many biomolecules, finally to increase lipid peroxides in the membrane (Smith et al 1996). Administration of (+)-catechin ameliorated the antioxidant activity of SOD and catalase in gastric mucosal tissue. However, (+)-catechin was reported to directly scavenge oxy free radical species in-vitro (Scott et al 1993; Nanjo et 1999). A marked reduction in ischaemia–reperfusion-induced mucosal damage coupled with a significant improvement in ulcer protection and gastric morphology contributed to its anti-ulcer effect, which may have been due to free radical scavenging activity of (+)-catechin. In addition, omeprazole scavenges the free oxygen radicals and has antioxidant effect (Bicakci et al 2005).

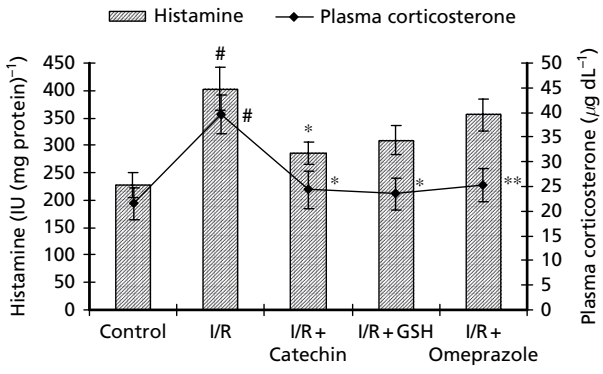

Figure 4 Effect of (+)-catechin, reduced glutathione (GSH) and omeprazole on I/R-induced histamine and corticosterone after 72-h I/R. Values are expressed as mean ± s.e.m. *$P < 0.05$ compared with I/R group. #$P < 0.01$ compared with control group.

Histamine is widely distributed in the gastrointestinal tract in various cells. It is involved in the pathogenesis of gastroduodenal ulceration, gastric inflammation and gastric acid secretion (Kitano et al 2005). Ischaemia alone rose the microdialysate histamine concentration for 30 min, which declined promptly by reperfusion for 60 min (Kitano et al 2005). Whereas, a significant decrease in gastric histamine content was observed immediately after ischaemia–reperfusion and this remained significantly reduced at 3 h after ischaemia–reperfusion. However, the mucosal histamine content rose significantly starting from 24 h after ischaemia–reperfusion and remained elevated. The plasma histamine concentration increased in the 72 h after ischaemia–reperfusion and this seemed to be related to the augmentation of gastric mucosal secretion and ulcer damage (Brzozowski et al 2000). Our observation indicated that treatment with (+)-catechin caused the reduction in histamine concentration in ischaemia–reperfusion animals, indicating the gastric defensive effect. On the other hand, H^+K^+ATPase showed a significant increase in mucosal concentrations in ischaemia–reperfusion rats. The H^+K^+ATPase is the dimeric enzyme responsible for H^+ secretion by the gastric parietal cells. The high levels of H^+K^+ATPase in ischaemia–reperfusion rats stimulate parietal cells to hypersecrete acid, which in turn causes the gastric ulcer and is selectively blocked by the action of omeprazole, an acid blocker used to treat gastric ulcers (Soumarmon et al 1983). However, (+)-catechin, a monomeric flavanol, in ischaemia–reperfusion injury significantly attenuated the elevated mucosal H^+K^+ATPase.

The level of alkaline phosphate, a brush-border enzyme, in ischaemia–reperfusion-induced gastric mucosal injury was significantly decreased compared with the control. This illustrated the increased mucosal permeability and a marked disturbance of the continuity of the gastric tissue (Young et al 1981). Nevertheless, (+)-catechin showed its activity by returning the alleviated mucosal alkaline phosphatase activity to the pre-ischaemic reperfusion level and repairing the gastric mucosa damage. The release of alkaline phosphatase suggested a role in tissue necrosis associated with polymorph neutrophil infiltration to the site of injury (Obi et al 2000). Further, more recent data have shown that neuro-endocrine immune axis is crucial during surgery (Filaretova et al 1998; Filaretova et al 2002). The degree of ulceration in ischaemia–reperfusion correlated positively with the level of plasma corticosterone. It is proposed that steroids, in quantities that the animal is capable of secreting, may contribute to the production of mucosal erosions and lead to ulcer (Weiss 1971). Thus, on the basis of these studies, the corticosterone increase during ischaemia–reperfusion was considered to be an ulcerogenic factor. However, (+)-catechin inhibited the plasma

corticosterone release during ischaemia–reperfusion-induced gastric ulcers. This suggested that (+)-catechin protected gastric mucosa against ischaemia–reperfusion-induced gastric ulcers by its antioxidant activity and gastric mucus protection.

Conclusion

The findings implied that the oral administration of (+)-catechin showed protective effects against ischaemia–reperfusion-induced gastric ulcer. Catechin is an antioxidant and may have quenched the free radicals responsible for ischaemia–reperfusion-induced stress in rats. We conclude that catechin, a component of green tea, is useful for the treatment of gastric ulcer.

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