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Epigallocatechin gallate accelerates healing of indomethacin-induced stomach ulcers in mice

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Abstract:
Management of the gastric toxicity of non-steroidal anti-inflammatory drugs (NSAIDs) remains a crucial problem because the commercially available drugs have side effects and are often expensive. Therefore, we examined the potential of the green tea-derived polyphenol epigallocatechin gallate (EGCG) to treat indomethacin-induced stomach ulcers in mice. Administration of indomethacin (18 mg/kg, *po*) to mice induced ulceration in the glandular portion of the gastric mucosa, accompanied by increased lipid peroxidation (LPO) and protein oxidation and reductions in thiol defense, mucin, cyclooxygenase (COX) expression and prostaglandin (PG) synthesis in the gastric tissues. Daily oral administration of EGCG (2 mg/kg) or omeprazole (3 mg/kg) for 3 days produced similar (~72–75%, $p < 0.001$) beneficial effects on the acute gastric ulceration. Treatment with the test samples partially reversed all the adverse oxidative effects of indomethacin. In addition, EGCG, but not omeprazole, enhanced expression of the COX isoforms and PG synthesis. The results suggest that the non-toxic and inexpensive tea polyphenol EGCG may be an excellent candidate for further evaluation as a potent anti-ulcer drug.

Key words:
antioxidant, COX, gastric ulcer, mucin, prostaglandin

Abbreviations: COX – cyclooxygenase, EGCG – epigallocatechin-3-gallate, MDS – macroscopic damage scores, PG – prostaglandin

Introduction

Widespread use of non-steroidal anti-inflammatory drugs (NSAIDs) has caused an alarming increase in the incidence of gastric, peptic, and even duodenal ulcers. Currently, the use of NSAIDs accounts for approximately 25% of gastric ulcer cases [18, 31]. In addition to causing gastric ulceration, NSAIDs also delay ulcer healing [16]. The currently prescribed synthetic anti-ulcer drugs are often expensive, have many side effects, and do not prevent ulcer recurrence [7, 34]. NSAIDs like indomethacin cause gastric ulcers through multiple mechanisms [37], including generation of ROS [36], neutrophil infiltration, cytokine imbalance, inhibition of prostaglandin (PG) synthesis [21], and initiation of lipid peroxidation [22, 36]. For decades, doctors have recommended dietary adjustments aimed at preventing or treating symptoms of

gastritis and ulceration, as diet may moderate the risk for gastritis or peptic ulcer [20].

Green tea is one of the most popular and widely consumed beverages. It is rich in a variety of catechin polyphenols such as (–)-epicatechin, (–)-epicatechin-3-gallate, (–)-epigallocatechin and (–)-epigallocatechin-3-gallate (EGCG). EGCG (chemical structure shown in Fig. 1), the most abundant tea polyphenol, is credited with anticancer, anti-diabetic and cardioprotective activities [4, 8]. Research has indicated that EGCG is a significantly more potent antioxidant than vitamin C and vitamin E and therefore may be more useful in the prevention and/or cure of various life-threatening diseases. Its anti-inflammatory properties have recently attracted attention [6], and EGCG capsules are currently sold as a nutraceutical at an affordable price. Reduced inflammation was observed in spontaneously colitic IL-2-deficient mice given green tea, and EGCG was suggested to be responsible for this effect [32]. Its efficacy against *Helicobacter pylori*-induced gastric toxicity has also been reported [19].

In view of these observations, we hypothesized that EGCG might be a useful nutritional, non-toxic agent for the treatment of NSAID-induced gastric ulcers. However, this aspect of EGCG has not yet been explored. In the present study, we evaluated its ability to heal indomethacin-induced acute gastric ulcers in mice and compared its efficacy with that of omeprazole. EGCG's healing activity correlated well with its ability to reduce the oxidative stress caused by indomethacin administration. EGCG was found to efficiently reduce lipid peroxidation, protein oxidation, and the depletion of thiol-dependent antioxidant defenses and mucin in gastric tissues. It also increased the expression levels of several cyclooxygenase isoforms and prostaglandin synthesis, thus accounting for faster ulcer healing.

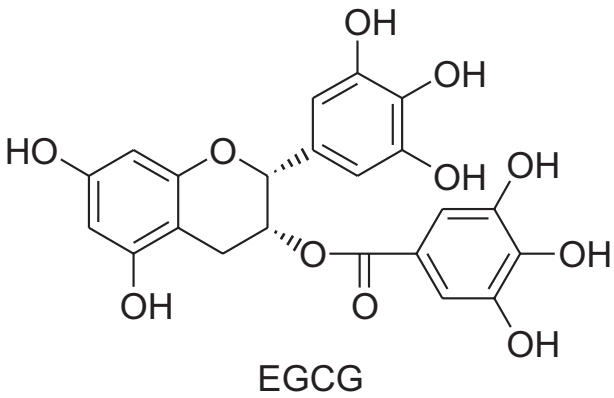

Fig. 1. Chemical structure of epigallocatechin gallate (EGCG)

Materials and Methods

Chemicals and reagents

Alcian blue, alum, bovine serum albumin (BSA), butylated hydroxytoluene (BHT), EGCG, eosin, guanidine hydrochloride, hematoxylin, indomethacin, omeprazole, nitrocellulose membrane, sucrose, trifluoroacetic acid (TFA), Tris-HCl and Tween 20 were procured from Sigma (St. Louis, MO). Other reagents used were 2-thiobarbituric acid (TBA), ethanol, butanol and ethyl acetate (all from E. Merck, Mumbai, India), trichloroacetic acid (TCA, Thomas Baker, Mumbai, India), hydrogen peroxide (35%, Lancaster, Morecambe, U.K.), 2,4-dinitrophenyl hydrazine (DNPH), disodium hydrogen phosphate and sodium dihydrogen phosphate (BDH, Mumbai, India), 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) (SRL, Mumbai, India), antibodies for COX-1 and COX-2 (Santa Cruz Biotechnology, Santa Cruz, CA), and a PGE metabolite EIA kit (Cayman Chem., Ann Arbor, MI).

Preparation of the test samples

Suspensions of EGCG and omeprazole in 2% gum acacia in water were prepared and orally administered to the mice.

Protocol for ulceration and healing studies

Male Swiss albino mice (25–30 g) bred at the BARC Laboratory Animal House Facility, Mumbai, India were procured after obtaining clearance from the BARC Animal Ethics Committee (BAEC). The animals were handled following International Animal Ethics Committee Guidelines, and the experiments were permitted by BAEC (sanction no. BAEC/09/07, dated 12.09.2008). The mice were reared on a balanced laboratory diet as prescribed by National Institute of Nutrition, Hyderabad, India and given tap water *ad libitum*. They were kept at 20 ± 2°C and 65–70% humidity with a 12 h day/12 h night periods. To perform all the experiments in a blinded fashion, all animals were identified by ear or nail notches and

randomized. Ulceration in the mice was induced by administering a single dose of indomethacin (18 mg/kg, *po*) dissolved in distilled water and suspended in 2% gum acacia. The animals were deprived of food (with free access to tap water) for 24 h before ulcer induction.

EGCG (0.5–5 mg/kg) was given to the mice once daily for up to 7 days; the first dose was given 6 h after indomethacin administration. On subsequent days, the test samples were given at 9 a.m. Omeprazole (3 mg/kg) was used as a positive control. The normal and ulcerated control groups of mice were given vehicle (0.2 ml) instead of drug for the entire period of the study. Each group had 5 mice, and each experiment was repeated three times. The mice were sacrificed on the third, fifth, and seventh days, 4 h after administering the last dose of the test samples. The extent of healing was assessed from the macroscopic damage scores (MDS) of the untreated and treated mice.

Assessment of ulcer healing as assessed by MDS

The mice were sacrificed after an overdose with thiopental. The stomachs from the normal and treated groups were removed rapidly, opened along the greater curvature, and thoroughly rinsed with normal saline. The ulcerated gastric mucosal areas were visualized with a dissecting microscope. The MDS was assessed [9] by grading the gastric injury on the following 0–4 scale, based on the severity of hyperemia and hemorrhagic erosions: 0 – almost normal mucosa, 0.5 – hyperemia, 1 – one or two lesions, 2 – severe lesions, 3 – very severe lesions, 4 – mucosa full of lesions (where "lesions" indicates hemorrhagic erosions and "hyperemia" indicates vascular congestions).

Histopathological and biochemical parameters of ulceration

The MDS results revealed peak ulceration and maximum ulcer healing by EGCG on the third day after indomethacin administration. Therefore, we assessed biochemical parameters on the third day of ulceration under the optimized doses of EGCG (2 mg/kg) and omeprazole (3 mg/kg). For this, the mice were equally divided into four groups, as follows:

Group I – normal mice; Group II – ulcerated mice; Groups III and IV – ulcerated mice treated with EGCG and omeprazole, respectively. The total antioxidant status (TAS) was determined from plasma, while the other biochemical parameters were analyzed using the ulcerated portions of the glandular stomach tissues of the mice. All experiments were carried out in triplicate with five animals per group, and data are presented as the mean ± SEM. The macroscopic data are presented from the review of a minimum of three sections per animal.

Histopathology

The ulcerated portions of the stomach were fixed in 10% formalin saline solution for 24 h, embedded in a paraffin block, and cut into 5 μm sections. These were placed on glass slides, stained with hematoxylin and eosin, and viewed under a light microscope. The macroscopic and histological experiments were performed by two investigators who were blinded to the group and treatment of animals. The histological sections were coded to eliminate an observer bias.

Quantification of protein and lipid damage

The glandular stomach tissues from five animals of each group were pooled and rinsed with phosphate buffer (50 mM, pH 7.4); the wet weight was then recorded. Tissues were homogenized in the same phosphate buffer with a glass–Teflon homogenizing tube and centrifuged at 1,200 × g to obtain the supernatant. The amount of protein carbonyls was determined using a previously described method [27]. Briefly, DNPH (4 ml, 10 mM) in 2 M HCl was added to the supernatant (1.0 ml), which was incubated for 1 h with intermittent shaking. An ice-cold 20% aqueous TCA solution (5 ml) was added, and the mixture was incubated for 15 min. The precipitated protein was washed three times with ethanol : ethyl acetate (1:1, v/v), dissolved in 1 ml of a solution containing 6 M guanidine HCl in 20 mM monobasic potassium phosphate, adjusted to pH 2.3 with TFA, and centrifuged. The amount of protein carbonyls was estimated from the absorbance of the supernatant at 362 nm ($\varepsilon = 2.2 \times 10^4\ M^{-1}\ cm^{-1}$).

For analyzing lipid peroxidation (measured in terms of thiobarbituric acid reactive species [TBARS]), a 10% homogenate of each tissue sample was prepared in a buffer containing 320 mM sucrose, 5 mM HEPES, 20 mM EDTA and 0.01% BHT. Samples were centrifuged at 1,200 × g for 15 min, and the supernatant was then centrifuged at 12,000 × g for 30 min to obtain mitochondrial pellets. The pellets were washed in 150 mM KCl/20 mM phosphate buffer and finally

suspended in phosphate buffer (50 mM, pH 7.4). The mitochondrial membrane fraction (1 ml) was treated with TCA/TBA/HCl (2 ml, 15% TCA, 0.375% TBA, 0.25 M HCl) containing 0.01% BHT, heated in a boiling water bath for 15 min, cooled, and centrifuged at 3,000 × g for 5 min. The amount of TBARS was estimated from the absorbance of the supernatant at 535 nm ($\varepsilon = 1.56 \times 10^5\ M^{-1}\ cm^{-1}$).

Measurement of non-protein thiol (NP-TSH)

Following a reported method [26], the fundic glandular stomach homogenates of different groups of mice were prepared in 0.2 M Tris-HCl buffer (pH 8.2) containing 20 mM EDTA and centrifuged at 1,200 × g for 15 min. The homogenate (1 ml) was treated with ice-cold 20% TCA (1 ml) and centrifuged at 3,000 × g for 5 min. The supernatant (1 ml) was added to Tris-HCl buffer (2 ml, 0.8 M, pH 9) containing 20 mM EDTA; DTNB (0.1 ml, 10 mM) was then added and the solution was thoroughly mixed. The NP-TSH content was estimated from the absorbance of the chromogen at 412 nm ($\varepsilon = 13.6 \times 10^4\ M^{-1}\ cm^{-1}$).

Quantification of TAS

Following a previously reported method [24] and the manufacturer's instructions, the plasma TAS level (mM/l) was measured using a Randox kit. Briefly, plasma (20 µl) or the standard (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, 1.65 mM/l) or reagent blank (double-deionized H_2O) was mixed with 1 ml chromogen (metmyoglobin, 6.1 µM/l and 2,2'-azino-di[3-ethylbenzthiazoline] sulfonate, 610 µM/l). After mixing, the initial absorbance (A1) at 600 nm was read at 37°C. Hydrogen peroxide (200 µl, 250 µM) was added to the sample, standard, or blank, and the absorbance (A2) was read after exactly 3 min. Subtraction of the A2 values from the respective A1 values gave the absorbance of the sample, standard, or blank. The respective TAS was obtained using the formula: TAS = factor × (absorbance of blank – absorbance of sample) mM/l; factor = concentration of standard/(absorbance of blank – absorbance of standard).

Mucin assay

Following a reported method [30], the free mucin content in the gastric tissues was estimated by measuring the amount of alcian blue bound to mucus. Briefly, the gastric tissues were incubated with a 1% buffered sucrose solution of 0.1% alcian blue in sodium acetate at 37°C for 60 min. After incubation, the mucosal tissues were washed with sucrose solution and centrifuged. The supernatant was extracted with $MgCl_2$, and the amount of alcian blue was estimated spectrophotometrically at 610 nm. The quantity of alcian blue (µg)/g of wet glandular tissue was calculated.

Western blot for cyclooxygenase expression

Equal amounts of tissue lysates (80 µg) were separated by 12% SDS-polyacrylamide gel electrophoresis and electrotransferred to nitrocellulose membranes. The membranes were blocked for 1 h at room temperature in TBST buffer (10 mM Tris–HCl, pH 8.0, 150 mM NaCl, and 0.1% Tween-20) containing 5% (w/v) nonfat milk, and incubated overnight at 4°C with the appropriate primary antibodies (1:3000). After several washes, HRP-conjugated secondary antibody (1:5000) was added, the membranes were incubated for 1 h, and the blots were developed using a Lumi-Light^{PLUS} western blotting kit. The bands were quantified and normalized to the β-actin bands using Kodak Gelquant software. The values (arbitrary units, the mean ± SEM) represent the density scanning results of three independent experiments, with the COX expression in normal mice set as 1.

PGE assay

The serum levels of PGE were assayed by ELISA, and the concentrations obtained are expressed in pg/ml.

Statistical analysis

The data are presented as the means ± SEM. The parametric data, which include all the biochemical parameters, were analyzed using a paired *t*-test for the paired data or a one-way analysis of variance (ANOVA) followed by a Dunnet multiple comparisons *post-hoc* test. Nonparametric data (macroscopic scores) were analyzed using a Kruskal-Wallis test (nonparametric ANOVA) followed by Dunn's multiple comparisons *post-hoc* test. A probability value of $p < 0.05$ was considered significant.

Results

Standardization of EGCG dose

For these experiments, we used doses of indomethacin (18 mg/kg) and omeprazole (3 mg/kg) that were chosen based on our earlier studies [2, 35]. The effective dose of EGCG was optimized by treatment with EGCG (0.5, 1, 2, 3, and 5 mg/kg) for up to seven days and comparing the MDS values of the treated and untreated mice on the respective days. The mice that received vehicle only showed no gastric lesion; however, lesions appeared within 6 h of indomethacin administration and reached a maximum on the third day after treatment. Thereafter, there was a gradual recovery due to natural healing, and on the seventh day after ulceration, the MDS value was reduced by 80% compared to the peak MDS value ($p < 0.001$) (data not shown). Treatment with EGCG dose-dependently accelerated the healing of gastric lesions. On the third day after ulceration, EGCG, at all doses tested, maximally reduced the MDS compared to the respective ulcerated control groups. Extending the treatment period improved the extent of healing, but most of it was due to natural healing. Therefore, for a better appreciation of the effect of the drugs, the MDS values on the third day of stomach ulceration with and without different doses of EGCG are shown in Table 1. On the third day of ulceration, optimal ulcer healing (~72%, $p < 0.001$) was obtained with EGCG (2 mg/kg); healing did not improve significantly at the higher doses (3 and 5 mg/kg). The effect of EGCG at high doses (2–5 mg/kg) was significantly better ($p < 0.01$) than that at lower doses (0.5 and 1 mg/kg). Under similar conditions, omeprazole (3 mg/kg) showed a similar effect on healing (75.2%, $p < 0.001$). During the entire study, the normal and ulcerated control groups of mice also received the vehicle (2% gum acacia in water) used for the administration of the test samples. The MDS values of these mice groups clearly revealed that gum acacia neither creates stomach ulcers nor produces any healing of indomethacin-mediated ulceration.

For the untreated mice, peak ulceration (maximum MDS) was observed on the third day of indomethacin administration. Hence, this time point was used to determine the IC_{50} value of EGCG. Considering the MDS values of the third day ulcerated untreated mice as 100%, the IC_{50} value of EGCG was found to be 2.94 ± 0.18 mg/kg (Fig. 2).

Tab. 1. Comparative healing capacities of EGCG at various doses and omeprazole at an optimized dose[a]

Groups	MDS values[b]	% Ulcer healing[c]
Untreated	3.43 ± 0.25	0
EGCG (0.5 mg/kg)	3.06 ± 0.19	10.79
EGCG (1 mg/kg)	1.84 ± 0.09*	46.36
EGCG (2 mg/kg)	0.91 ± 0.08**,†	71.43
EGCG (3 mg/kg)	0.84 ± 0.07**,†	75.51
EGCG (5 mg/kg)	0.63 ± 0.05**,†	81.63
Omeprazole (3 mg/kg)	0.85 ± 0.09**	75.21

[a] Stomach ulceration in mice was induced by oral gavage of indomethacin (18 mg/kg). Treatment was carried out for 3 days with different doses of ECGC and an optimized dose of omeprazole (3 mg/kg). [b] The MDS values (the mean ± SEM of three independent experiments, each with 5 mice per group) were measured on the third day after indomethacin administration. [c] Healing was calculated and normalized to the MDS value for the ulcerated, untreated mice, which was set as 100%. * $p < 0.01$, ** $p < 0.001$ compared to the ulcerated mice; † $p < 0.01$ compared to EGCG (0.5 and 1 mg/kg) treatment

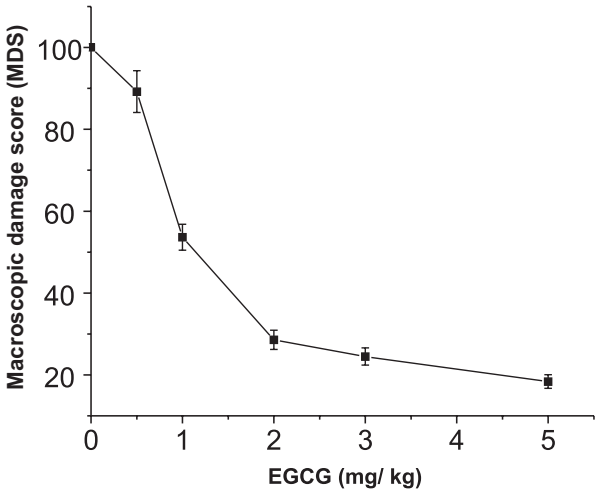

Fig. 2. Dose-dependent effects of EGCG in indomethacin (18 mg/kg, *po*)-induced stomach ulcers in mice after 3 days of treatment. Healing was assessed by measuring the MDS values (the mean ± SEM of three independent experiments, 5 mice/group) 4 h after the last dose of EGCG, which were normalized to the MDS value of the group II mice. The IC_{50} value for EGCG was determined by probit analysis of the data

Histopathological assessment of ulcer healing by EGCG

Histological photographs of stomach sections (Fig. 3) of normal, ulcerated, and treated mice revealed that indomethacin caused marked damage to the glandular portion of the gastric mucosa. Within 6 h after indo-

Fig. 3. Histological assessment of the acute gastric mucosal injury induced by indomethacin (18 mg/kg, *po*) in mice and its prevention by EGCG (2 mg/kg, *po* × 3 days) and omeprazole (3 mg/kg, *po* × 3 days). Stomach sections of normal, ulcerated and treated mice were prepared 4 h after the last dose of the drugs on the third day of ulceration and processed for visualization. The arrow indicates superficial mucosal damage

methacin administration, superficial erosion and mild inflammation in the stomach were observed, indicating acute ulceration (data not shown). Multiple punched-out areas of ulceration in the mucosa that contained inflammatory infiltrate containing neutrophils and macrophages, along with hemorrhagic serosa, were evident on the third day of ulceration. Treatment with EGCG or omeprazole for 3 days reduced the number of inflammatory cells and mucosal congestion while increasing the number of healthy normal cells in the gastric mucosa, submucosa, serosa and muscle layers. Mucosal hyperplasia and cryptic proliferation with no frank denudation were the main hallmarks of the treatment. The effect of EGCG was slightly better than that of omeprazole.

Effect of EGCG and omeprazole on oxidation of lipids, proteins, and NP-TSH

Indomethacin administration markedly stimulated lipid peroxidation in gastric tissues, and the TBARS content was elevated 2 fold ($p < 0.001$) compared to that of normal mice. EGCG and omeprazole reduced ($p < 0.05$) TBARS content by 34.6% and 27.6%, respectively, compared to the group II mice. The protein carbonyl content of the ulcerated mouse tissue was also increased (> 2.1 fold, $p < 0.001$), compared to the normal value, and reduced by EGCG and omeprazole ($p < 0.01$) by 40.3% and 35.2%, respectively. Ulceration also decreased gastric NP-TSH significantly (62%, $p < 0.01$), compared to the normal value. Treatment with EGCG and omeprazole increased ($p < 0.01$) the

Tab. 2. The effects of EGCG and omeprazole on the levels of some biochemical parameters in the ulcerated gastric tissue of mice on the third day after ulceration[a]

Parameters	Normal	Ulcerated Untreated	EGCG-treated	Omeprazole-treated
TBARS (nmol/mg protein)	1.26 ± 0.19	2.57 ± 0.39***	1.68 ± 0.16$^{\#}$	1.86 ± 0.21$^{\#}$
Protein carbonyls (nmol/mg protein)	1.39 ± 0.16	2.98 ± 0.32***	1.78 ± 0.18†	1.93 ± 0.17†
NP-TSH (nmol/mg tissue)	2.58 ± 0.12	1.6 ± 0.2**	2.54 ± 0.33†	2.41 ± 0.41†
TAS (mM)	1.36 ± 0.13	0.64 ± 0.15***	1.09 ± 0.11††	1.01 ± 0.09†
Mucin (µg/g tissue)	232.81 ± 17.61	117.61 ± 22.19*	215.4 ± 9.67††	219.2 ± 16.39††
PGE (pg/ml)	263.25 ± 5.86	78.52 ± 18.81***	162.97 ± 19.67$^{\dagger\dagger,\$}$	111.90 ± 14.21†

[a] Stomach ulceration in mice was induced by indomethacin (18 mg/kg, *po*). Treatment was carried out with EGCG (2 mg/kg, *po*) and omeprazole (3 mg/kg, *po*) for 3 days. The assays were carried out 4 h after the last dose of the drugs, and the values represent the mean ± SEM of three independent experiments, each with 5 mice per group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, compared to normal mice; $^{\#}$ $p < 0.05$, † $p < 0.01$, †† $p < 0.001$, compared to ulcerated mice; $^{\$}$ $p < 0.01$, compared to omeprazole-treated mice

NP-TSH levels by 58.8% and 50.6% ($p < 0.01$), respectively, compared to those in the untreated mice. These results are summarized in Table 2.

Effects of EGCG and omeprazole on plasma TAS

Compared to the normal mice, a significant decrease (52.6%, $p < 0.001$) in the plasma TAS level was noticed on day three of ulceration. However, treatment with EGCG and omeprazole increased the TAS level by 69% ($p < 0.001$) and 57.9% ($p < 0.01$), respectively, compared to that of the group II mice (Tab. 2).

Effects of EGCG and omeprazole on gastric mucin

Compared to the normal value, ulceration reduced the mucin level by 49.8% ($p < 0.01$). Treatment with EGCG and omeprazole restored it to near normalcy ($p < 0.001$). The results are presented in Table 2.

Effects of EGCG and omeprazole on COX enzymes

The western blots of COX-1 and COX-2 expressions in the gastric mucosa of the normal, ulcerated and drug (EGCG and omeprazole)-treated mice are shown in Figure 4. The blot of normal gastric tissues showed very strong COX-1 expression, with a low intensity band for COX-2. The indomethacin-induced gastric ulceration depleted ($p < 0.001$) the expression of COX-1 and COX-2 by 72% and 33%, respectively. Treatment with EGCG increased both COX-1 (2.6 fold, $p < 0.001$) and COX-2 (3.6 fold, $p < 0.001$), compared to that of the untreated mice. Omeprazole also increased COX-1 (2.4 fold, $p < 0.001$) and COX-2 (2.6 fold, $p < 0.001$), compared to the ulcerated group. The effects of EGCG and omeprazole were significantly different for COX-2, but not for COX-1.

Fig. 4. Immunoblots of COX-1 and COX-2 expressions in the stomach tissues of normal, ulcerated and treated mice. Ulceration was induced by indomethacin (18 mg/kg, *po*). Mice were treated for 3 days with EGCG (2 mg/kg, *po*) or omeprazole (3 mg/kg, *po*). The bands were normalized to β-actin bands and then to the value for normal mice (set as 1) using Kodak Gelquant software. The values (arbitrary units, the mean ± SEM) represent the densitometric scanning results of three independent experiments

Effect of EGCG and omeprazole on serum PGE levels

The serum PGE level was reduced by 70.2% ($p < 0.001$) on the third day of ulceration, compared to that of normal mice (Tab. 2). Treatment with ECGC increased the PGE concentration 2.1 fold ($p < 0.001$), compared to that of the group II mice. Its effect was significantly better ($p < 0.01$) than that of omeprazole (42.5% increase, $p < 0.01$).

Discussion

Ulcer healing is a complex process involving various factors. The gastric toxicity of NSAIDs like indomethacin can be attributed to their ability to induce reactive oxygen metabolites and reduce PG synthesis by inhibition of the COX isozymes. In addition, the NSAIDs produce hemorrhagic ulcer by decreasing gastric mucus production [25]. After acute injury, release of preformed mucus promotes epithelial recovery by forming a mucoid cap beneath which reepithelization occurs [28]. The mucus offers protection against endogenous threats like acid, pepsin, and oxidants, produced in the gastric lumen, as well as exogenous agents such as NSAIDs. Thus, drugs that can arrest ulcer progression by acting as antioxidants, increasing the synthesis and secretion of gastric mucus, and promoting PG synthesis should accelerate gastric ulcer healing.

Our macroscopic and histopathological results showed marked gastric mucosal damage in mice on the third day after indomethacin administration. This led to elongated hemorrhagic lesions, which were confined to the glandular portion, and the highest subjective ulcer score seen in this study. The natural heal-

ing observed in the untreated mice revealed that the ulceration was acute. However, the natural healing was much slower in the control than in the treated groups. The efficacy of EGCG (2 mg/kg) was similar to that of omeprazole (3 mg/kg).

Stomach ulceration is also associated with oxidative damage to lipids, proteins and the thiol-dependent antioxidant defense system. This was apparent from the increased accumulation of TBARS and protein carbonyls as well as depletion of NP-TSH in the gastric tissues of the ulcerated mice. Excessive lipid peroxidation and accumulation of H_2O_2 can cause increased consumption of glutathione, as reported earlier for indomethacin-induced gastropathy [1, 3]. Sulfhydryl compounds help recycle endogenous antioxidant vitamins and prevent lipid peroxidation. More importantly, they also protect mucus by preventing rupture of the disulfide bridges that join the mucus subunits and maintain structural integrity [5]. The decrease in endogenous thiol (glutathione) upon ethanol-induced gastric injury and its role in mucosal protection has previously been demonstrated [23]. Both EGCG and omeprazole significantly and to similar extents suppressed oxidative damage to biomacromolecules. This could decrease ulcer progression and promote healing of gastric lesions induced by acute indomethacin treatment.

Compared to the individual oxidative markers, assay of the plasma TAS level provides a better index of the body's total systemic antioxidant defense; it is a marker for activity of enzymes such as superoxide dismutase and the selenium-containing glutathione peroxidase as well as nonenzymatic antioxidants (radical scavengers and chelating agents) and of their synergistic interaction [10]. Our plasma TAS level assays revealed severe oxidative stress in gastric tissue of the indomethacin-treated mice. The compounds tested both improved this parameter markedly, though EGCG was more potent than omeprazole.

Besides oxidative stress, mucosal damage can also be produced by depletion of gastric mucosal mucin. Maintenance of mucus production may provide partial but significant protection against reactive oxygen metabolites. Our results revealed that stomach ulceration decreased the gastric mucin content. This could reduce the ability of the mucosal membrane to protect the mucosa from physical damage and damage due to back diffusion of hydrogen ions. Treatment with EGCG and omeprazole restored the gastric mucus layer to near normalcy.

The NSAIDs exert both their therapeutic and toxic effects mainly by decreasing the levels of circulating PGE at the gastric mucosa *via* inhibition of the cyclooxygenases. The reduced level of PGs is known to cause gastric ulceration and exacerbate pre-existing gastric ulcers in rodents and humans [13, 33]. Besides stimulating mucus and bicarbonate secretion and mucosal blood flow, PGs also contribute to ulcer healing by inducing angiogenesis [17]. Our immunoblots revealed reduced expressions of COX-1 and COX-2 at peak ulceration, leading to reduced PGE synthesis. Treatment with EGCG increased the expressions of both the enzymes, although the effect was significantly greater for COX-2. In line with these results, the concentration of serum PGE was also increased. Enhanced PG synthesis is known to stimulate EP4 receptor-mediated mucin synthesis [15] and inhibit neutrophil-mediated free radical generation [12]. Therefore, stimulation of PGE expression by EGCG might contribute to the antioxidative and mucin modulatory properties observed in the present study. Consistent with an earlier report, we did not observe any anti-secretory effect of EGCG [14].

Although omeprazole also increased the expressions of the COX isoforms, it did not greatly increase PGE synthesis. This suggests that the beneficial properties of omeprazole might be primarily due to its ability to control intragastric pH [11] and stimulate epithelial cell proliferation *via* an increase in the serum gastrin level [29]. In addition, its antioxidant action and pro-mucin effects may contribute to its healing properties.

Overall, the green tea-derived nutraceutical EGCG was able to promote healing of indomethacin-induced gastric ulcers in mice. This result is corroborated by comparison of EGCG's effects with those of the positive control omeprazole. The beneficial effects of EGCG and omeprazole were apparently due to their antioxidant actions and ability to form mucin. Additionally, EGCG augmented PG synthesis by upregulation of the COX isozymes, which may also contribute to ulcer healing. Because it is non-toxic, EGCG appears to be a promising anti-ulcerogenic agent for further evaluation. Because of the importance of angiogenesis in ulcer healing, it would be interesting to study the effect of EGCG on pro- and anti-angiogenic factors; such investigations are currently under way in our laboratory.

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