Pharmacological Reports
2011, 63, 527–536
ISSN 1734-1140

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

Biplab Adhikary[1], Sudhir K. Yadav[1], Sandip K. Bandyopadhyay[1], Subrata Chattopadhyay[2]

[1]Department of Biochemistry, Dr. B.C. Roy Post Graduate Institute of Basic Medical Sciences & IPGME&R, 244B, Acharya Jagadish Chandra Bose Road, Kolkata – 700 020, India

[2]Bio-Organic Division, Bhabha Atomic Research Centre, Mumbai – 400 085, India

Correspondence: Subrata Chattopadhyay, e-mail: schatt@barc.gov.in

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.

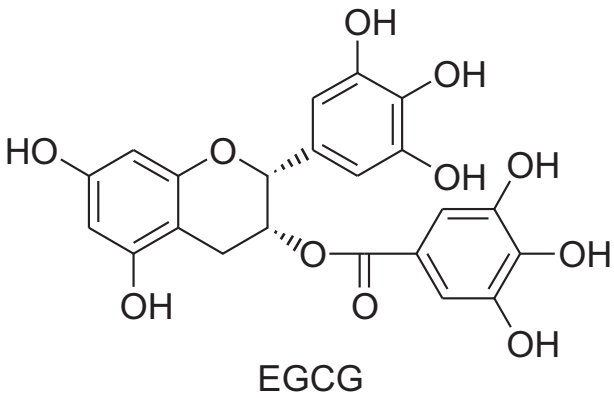

Fig. 1. Chemical structure of epigallocatechin gallate (EGCG)

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