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Green tea polyphenol epigallocatechin-3-gallate shows therapeutic antioxidative effects in a murine model of colitis ☆

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Abstract

Background and aims: Leukocyte infiltration, up-regulation of proinflammatory cytokines and severe oxidative stress caused by increased amounts of reactive oxygen species are characteristics of inflammatory bowel disease. The catechin (2R,3R)-2-(3,4,5-Trihydroxyphenyl)-3,4-dihydro-1(2H)-benzopyran-3,5,7-triol-3-(3,4,5-trihydroxybenzoate), named epigallocatechin-3-gallate, EGCG, has been demonstrated to exert anti-inflammatory and antioxidative properties, reducing reactive oxygen species in the inflamed tissues. The aim of this study was to evaluate the therapeutic effects of EGCG in a murine model of colitis induced by oral administration of dextran sodium sulfate.

Abbreviations EGCG, Epigallocatechin-3-gallate; DSS, dextran sodium sulfate; ROS, reactive oxygen species; SOD, superoxide dismutase; CAT, catalase; GPO, gluthathione peroxidase; ERK, extracellular signal-regulated kinase; MPO, myeloperoxidase; MDA, malondialdehyde; TBA, thiobarbituric acid.

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Methods: Mice received a daily oral administration of 6.9 mg/kg body weight EGCG or *Piper nigrum* (L.) alkaloid (2E,4E)-5-(1,3-benzodioxol-5-yl)-1-piperidin-1-ylpenta-2,4-dien-1-one, named piperine (2.9 mg/kg body weight) or the combination of the both — piperine was used in this combination to enhance the bioavailability of EGCG.
Results: *In vivo* data revealed the combination of EGCG and piperine to significantly reduce the loss of body weight, improve the clinical course and increase overall survival in comparison to untreated groups. The attenuated colitis was associated with less histological damages to the colon and reduction of tissue concentrations of malondialdehyde, the final product of lipid peroxidation. Neutrophils accumulation indicator myeloperoxidase was found to be reduced in colon tissue, while antioxidant enzymes like superoxide dismutase and glutathione peroxidase showed an increased activity. *In vitro*, the treatment with EGCG plus piperine enhanced the expression of SOD as well as GPO and also reduced the production of proinflammatory cytokines.
Conclusion: These data support the concept of anti-inflammatory properties of EGCG being generally beneficial in the DSS-model of colitis, an effect that may be mediated by its strong antioxidative potential.

1. Introduction

A combination of various etiological factors of inflammatory bowel disease (IBD) eventually leads to mucosal breakdown and ulcerations in active states of IBD. [1] The inflammation process includes a massive infiltration of polymorpho- and mononuclear phagocytic leukocytes producing large amounts of proinflammatory mediators, one of them being reactive oxygen species (ROS). Superoxide anion, hydrogen peroxide, and hypochlorous acid consist of highly reactive molecules as a result of the presence of unpaired electrons. [2,3] Due to dietary factors, the intestinal microflora, the large exposure to the outside environment and the interactions between cells of the immune system, the bowel is a major site of oxidant entry and production. [4]

Recent studies have examined catechin (−)-epigallocatechin-3-gallate (EGCG), [5,6] which represents up to 30% of the dry weight of green tea leaves. [7] Catechins have been shown to display antioxidant and anti-inflammatory properties *in vitro*. In animal models, [8,9] they have been shown to be more effective antioxidants compared to vitamins E and C. [10] This antioxidative and radical scavenging activity as shown *in vitro* and *in vivo* [11,12] can be attributed to the presence of the phenolic hydroxyl groups on the B- and D-rings of the catechin molecule. [13] Recent studies applied EGCG intraperitoneally, using doses up to 50 mg/kg bodyweight. In the present study, we aimed at further investigating the antioxidant potential of EGCG combining it with a second dietary component, 1-piperoylpiperidin (piperine), an alkaloid from *Piper nigrum* (L.) (Piperaceae family). In order to correspond to applicable forms of intake even for humans, we administered EGCG intragastrically. After intragastric application, EGCG shows a low bioavailability and significant biotransformation leading to a reduction of the effective compound. [14] Extensive metabolism leads to the formation of glucuronidated, sulfated and methylated conjugates. [15] Uptake into enterocytes is thought to be dependant on membranous saturable monocarboxylate transporters (MCT), while cellular EGCG is being actively effluxed across the apical membrane by multidrug resistance-related protein (Mrp) 1 and 2 and to some extent by P-glycoprotein, a fact that may limit absorption of EGCG from the gut and its availability to the plasma. [16,17] The absolute bioavailability of EGCG in mice models is reported to be as much as 26.5%. [18]

Therefore, it seemed necessary to increase the low bioavailability of EGCG after intragastrical application by coadministration of piperine effecting reduced small intestinal glucuronidation by 40 to 60% and increasing intestinal tissue and systemic concentrations of free EGCG. [19]

Being continually exposed to ROS, the body has developed several endogenous antioxidant defense mechanisms: enzymes like superoxide dismutases (SOD), gluthathione peroxidases (GPO) and low molecular weight antioxidants such as vitamin C or vitamin E. [20] A deficiency in GPO genes for example leads to symptoms and pathology of IBD in mice. [21]

However, the very imbalance between the increased production of ROS and the decreased detoxification by antioxidants initiates inflammatory cascades by upregulating different genes involved in the inflammatory response. [22,23] Furthermore, large amounts of ROS result in the damage of cellular proteins, [24] lipids, [25] cytoskeleton, [26] even DNA and ultimately, disruption of gastrointestinal barrier integrity with an increased gut permeability. [27,28] This appears to be a major pathogenic mechanism in IBD, [29] as chemiluminescence assays of samples from the colonic mucosa of patients with ulcerative colitis (UC) as well as animal models of UC show increased levels of ROS in active disease. [30,31]

EGCG further interferes with other steps of the inflammatory process, e.g. it inhibits the secretion of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and IL-8 through the attenuation of extracellular signal-regulated kinases (ERK) and NF-κB in human mast cell lines (HMC-1). [32] Furthermore, EGCG influences a number of signaling pathways, including activator protein 1 (AP-1) or the synthesis of eicosanoids and prostaglandin E_2 (PGE_2). [33] EGCG also shows anticarcinogenic effects in epidemiological and animal studies; administration of green tea, green tea extract or EGCG reduced tumor formation and growth and showed antiangiogenic and antimutagenic properties. [34–36]

The objective of the present study was to address whether EGCG exerts protection on DSS-induced chronic colitis in the