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Epigallocatechin gallate supplementation protects against renal injury induced by fluoride intoxication in rats: Role of Nrf2/HO-1 signaling

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ABSTRACT

Fluoride intoxication generates free radicals, causing oxidative stress that plays a critical role in the progression of nephropathy. In the present study, we hypothesized that epigallocatechin gallate (EGCG), found in green tea, protects the kidneys of rats treated with fluoride by preventing oxidative stress, inflammation, and apoptosis. Pretreatment of fluoride-treated rats with EGCG resulted in a significant normalization of creatinine clearance and levels of urea, uric acid, and creatinine. Fluoride intoxication significantly increased renal oxidative stress markers and decreased the levels of renal enzymatic and non-enzymatic antioxidants. In addition, renal NO, TNF-α, IL-6 and NF-κB were also increased in the renal tissue of fluoride-treated rats. Further, EGCG pretreatment produced a significant improvement in renal antioxidant status and reduced lipid peroxidation, protein carbonylation and the levels of inflammatory markers in fluoride-treated kidney. Similarly, mRNA and protein analyses showed that EGCG pretreatment normalized the renal expression of Nrf2/Keap1 and its downstream regulatory proteins in fluoride-treated rat kidney. EGCG also effectively attenuated fluoride-induced renal apoptosis by the up-regulation of anti-apoptotic proteins such as Bcl-2 and down-regulation of Bax, caspase-3, caspase-9 and cytochrome c. Histology and immunohistochemical observations of Kim-1 provided further evidence that EGCG effectively protects the kidney from fluoride-mediated oxidative damage. These results suggest that EGCG ameliorates fluoride-induced oxidative renal injury by activation of the Nrf2/HO-1 pathway.

Abbreviations: ATPase, adenosine triphosphatase; Bcl-2, B-cell lymphoma 2; Bax, B-cell associated X protein; CAT, catalase; EDTA, ethylenediaminetetraacetic acid; EGCG, epigallocatechin gallate; GAPDH, glyceraldehyde 3 phosphate dehydrogenase; GCSH, γ-glutamylcysteine synthetase heavy subunit; G6PD, glucose 6-phosphate dehydrogenase; GPx, glutathione peroxidase; GR, glutathione reductase; GST, glutathione *S*-transferease; GSTM, glutathione *S*-transferase Mu; HO-1, heme oxygenase-1; IL-6, interleukin-6; Keap-1, Kelch-like ECH-associated protein 1; Kim-1, kidney injury molecule-1; LOOH, lipid hydroperoxide; NaF, sodium fluoride; NF-kB, Nuclear factor kappa B; Nrf2, nuclear factor erythroid-2 related factor-2; PC, protein carbonyl; ROS/RNS, reactive oxygen species/reactive nitrogen species; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances; TNF-α, tumor necrosis factor-α; TSH, total sulfhydryl groups.

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1. Introduction

Fluoride is used in numerous industrial activities and is a ubiquitous component of water sources, foods, and dental products [1]. Fluoride ions can easily spread from the lungs and the gastrointestinal tract to the blood. Numerous disorders have been connected to systemic fluoride consumption [2]. Fluoride compounds are used to enhance the strength of teeth, for water fluoridation and as food additives [3,4]. The minimal risk level for daily oral fluoride uptake was determined to be 0.05 mg/kg/day, based on a non-observable adverse effect level (NOAEL) of 0.15 mg Fl/kg/day for an increased fracture rate. Estimations of human lethal fluoride doses showed a wide range of values, from 16 to 64 mg/kg in adults and 3 to 16 mg/kg in children [3]. Skeletal and dental fluorosis are the most common diseases caused by excessive consumption of fluoride. In advanced cases, skeletal fluorosis causes pain and damage to bones and joints [5]. In India, the most common cause of fluorosis is excessive consumption of fluoride-laden water derived from the deep bore wells. Over half of the ground water sources in India have fluoride above the levels recommended by the WHO [6].

Fluoride enhances the generation of reactive oxygen species and decreases antioxidant enzyme capacity, effects that play critical roles in fluoride-induced organ toxicity [7–9]. Natural antioxidants can help to conquer the oxidative stress and free radical-induced disorders. Numerous side effects of synthetic antioxidants have been reported previously. Therefore, attention has been recently paid to find natural antioxidants with lower side effects. Green tea catechins, especially epigallocatechin gallate (EGCG), are known to be the most potent antioxidant among all catechins [10]. Epidemiological and intervention studies indicate that consumption of 5–6 or more cups of green tea, containing 200–300 mg EGCG, per day may be beneficial for maintaining normal health status [11]. EGCG acts as a scavenger of many reactive oxygen/nitrogen species (ROS/RNS) such as superoxide radical anion, peroxyl and hydroxyl radicals, singlet oxygen, nitric oxide and peroxynitrite. EGCG may trap peroxyl radicals and thus break the chain reaction of free radicals and terminate lipid peroxidation. An electron paramagnetic resonance (EPR) study on tea catechins indicated that each molecule of EGCG has the ability to trap six superoxide anion or hydroxyl radicals [12].

EGCG has already been reported as a beneficial agent to manage the development of diabetic nephropathy and chronic renal failure induced by streptozotocin and adenine, respectively [13]. The potential role of oxidative stress in the renal injury associated with fluoride exposure suggests that inhibition of ROS-mediated oxidative stress may protect against fluoride-induced nephrotoxicity. These findings led us to hypothesize that EGCG might reduce fluoride-induced oxidative stress and inflammation and provide a defensive mechanism against fluoride nephrotoxicity. Thus, the purpose of the present study was to investigate the possible protective role of EGCG on fluoride-induced nephrotoxicity and to explore the underlying molecular pathways involved in the nephroprotection rendered by EGCG.

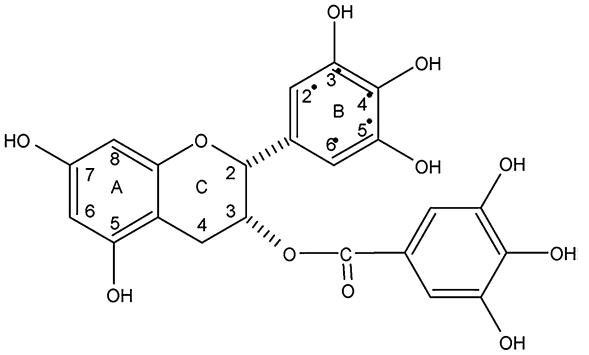

Fig. 1. Chemical structure of EGCG ($C_{22}H_{18}O_{11}$).

2. Materials and methods

EGCG was purchased from Sigma Aldrich (St. Louis, MO, USA). The antibody against Nrf2 was procured from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibody against heme oxygenase-1 (HO-1) was purchased from Abcam (Cambridge, UK). Goat anti-Bcl-2, Bax, cytochrome c, cleaved caspases 9 and 3, and β-actin monoclonal antibodies were purchased from Santa Cruz Biotechnology. Anti Kim-1 rat polyclonal antibody was purchased from Thermo Scientific (Pittsburgh, PA, USA). Sodium Fluoride (NaF, molecular weight 41.98) and all other chemicals and solvents were of certified analytical grade and purchased from S.D. Fine Chemicals (Mumbai, India) or Hi Media Laboratories Pvt. Ltd. (Mumbai, India). Reagent kits were obtained from Span Diagnostics (Mumbai, India). The structure of EGCG is shown in Fig. 1.

2.1. Animals and diet

Healthy male albino Wistar rats (160–180 g) were obtained from the Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University, and maintained in an air-conditioned room (25 ± 2 °C) with a 12 h light/12 h dark cycle. Food and water were provided ad libitum to all the animals. The study protocols were approved by the Institutional Animal Ethics Committee of Rajah Muthiah Medical College and Hospital (Reg No. 160/1999/CPCSEA, Proposal number: 952/2012), Annamalainagar and the study conducted in accordance with the Guide for the Care and Use of Laboratory Animals.

2.2. Experimental design

The rats were maintained under standard laboratory conditions (temperature 24 ± 2 °C; natural light–dark cycle). The rats had free access to drinking water and commercial standard pellet diet (Lipton India Ltd., Mumbai, India). In the present study, fluoride was administered intragastrically at a dose of 25 mg/kg body weight/day for 4 weeks, which was 1/10 of the oral LD_{50} value [14] in rats. The dose selection for EGCG (40 mg/kg) was based on the previous report of Thangapandiyan and Miltonprabu [15], which showed this dose to be protective against