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Chlorogenic acid ameliorates experimental colitis in mice by suppressing signaling pathways involved in inflammatory response and apoptosis

Iva Vukelić[1], Dijana Detel[1], Lara Batičić Pučar, Iva Potočnjak, Sunčica Buljević, Robert Domitrović*

Department of Chemistry and Biochemistry, Faculty of Medicine, University of Rijeka, Rijeka, Croatia

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ABSTRACT

Chlorogenic acid (ChA) exhibits a multitude of positive health effects, however, the signaling mechanisms by which ChA could influence the inflammatory response in experimental colitis are unknown. To answer this question, we induced colitis in mice by administration of 2.5% dextran sulfate sodium (DSS) in drinking water for seven days. Oral administration of ChA significantly ameliorated clinical symptoms, improved disease activity index and colon shortening induced by DSS. Furthermore, ChA administration resulted in a suppression of phosphorylated extracellular signal-regulated kinases 1 and 2 (ERK1/2), c-Jun N-terminal kinases 1 and 2 (JNK1/2), Akt and signal transducer and activator of transcription 3 (STAT3) with concomitant upregulation of phosphatase and tensin homolog (PTEN) expression. Immunohistochemical analysis showed a dose-dependent decrease in expression and nuclear translocation of nuclear factor-kappa B (NF-κB) p65 subunit, which was accompanied by suppression of pro-inflammatory cytokine tumor necrosis factor-alpha (TNF-α) expression. Induction of apoptosis and oxidative stress was attenuated in a dose-dependent manner by suppressing Bax, caspase-8, caspase-9 and heme oxygenase-1 (HO-1) protein expression in mice administrated with ChA. The results of the current study suggest that ChA could be useful for the treatment of inflammation and attenuating colitis severity by suppressing activation of pro-inflammatory and apoptotic signaling pathways.

1. Introduction

Ulcerative colitis (UC) is one of the chronic inflammatory bowel diseases (IBD) of unknown etiology with a relapsing and remitting course that severely distresses the quality of life of patients. It is characterized by recurring episodes of inflammation that almost always involve the rectum and can extend proximally in a continuous manner, affecting the mucosal layer of the large bowel (Vanga and Long, 2018). Besides continuous efforts to clarify the ethiopathogenesis of UC and invested energies in findings and inventions of new therapeutic strategies, this disease remains a challenge in medical and scientific community. In order to obtain better insights into molecular events which are crucial for the disease development, as well as in order to find new therapeutic possibilities, different animal models of UC were developed. The chemically-induced dextran sulfate sodium (DSS) colitis model has been shown to resemble human UC in many different aspects of disease pathology, from abnormal immune response to clinical manifestation (Detel et al., 2016).

Mitogen-activated protein kinases (MAPKs) represent a family of cellular enzymes which form one of the best studied signal transduction pathway. For three major constituents of the MAPKs family, extracellular signal-regulated kinases (ERKs), c-Jun N-terminal kinases (JNKs) and p38 MAPKs families are well known to be activated in response to a diverse range of stimuli, playing a crucial role in many inflammatory disorders including IBD (Hommes et al., 2003). Likewise, previously published data suggest that the phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway plays a significant role in the development and progression of UC. Therefore, the inhibition of PIK3 has been suggested as a possible drug candidate for the treatment of UC (Huang et al., 2011). Interestingly, available data suggest that signal transducer and activator of transcription 3 (STAT3) has both positive and negative effects on the progression of colon inflammation. STAT3 inhibition in T cell reduced T cell-mediated intestinal inflammation (Atreya et al., 2000). However, activation of STAT3 in colonic epithelial cells has been shown to have a protective role (Takeda et al., 1999). The nuclear factor-kappa B (NF-κB) (NF-κB) signaling pathway is another pathway involved in the pathogenesis of inflammatory diseases such as intestinal inflammation (Zhang et al., 2017; Wullaert et al., 2011).

* Corresponding author. Department of Chemistry and Biochemistry, Faculty of Medicine, University of Rijeka, B. Branchetta 20, 51000, Rijeka, Croatia.
E-mail address: robert.domitrovic@medri.uniri.hr (R. Domitrović).
[1] Authors contributed equally to this work.

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Animal models as well as epidemiological evidence reveals a positive causal connection between consumption of phytochemical compounds contained in healthy food and alleviation of chronic degenerative diseases, including UC (Tan and Zheng, 2018; Albini et al., 2017). Chlorogenic acid (ChA), as one of the most abundant polyphenol in medicinal plants as well as food and beverages, has been demonstrated to have very valuable actions including anti-inflammatory, antioxidant, anti-microbial and anti-carcinogenic properties (Naveed et al., 2018). However, despite the multifunctionality of ChA, little is known about its role in UC and especially the mechanisms of action in inflamed tissue. According to the current knowledge, the beneficial effects of ChA in amelioration of colon tissue inflammation could be suggested since the anti-oxidative and anti-inflammatory effects of ChA were shown (Shin et al., 2015). However, there are no data regarding the role of MAPKs, STAT3 or PI3K/Akt signaling in the mechanism of protective activity of ChA in experimental colitis.

Therefore, the aim of this study was to characterize and clarify mechanisms by which ChA could influence on colitis development and inflammatory changes present in DSS-induced colitis. This study aims to provide further justification for the use of ChA in treating inflammatory disorders such as UC by further delineating the involvement of the signaling cascades in the inflammatory response.

2. Material and methods

2.1. Animals

Male, 8-10-week-old C57BL/6 mice were purchased from the Central Animal Facility of the Faculty of Medicine, University of Rijeka, Croatia. Mice were housed at 23 ± 2 °C and acclimated for 7 days. Laboratory animals were fed with standard pellet food (MK, Complete Diet for Laboratory Rats and Mice, Slovenia) and given tap water ad libitum. All animal treatments were approved by the Ethical Committee of the Faculty of Medicine University of Rijeka and were performed in accordance with the Croatian Law for the Protection of Laboratory Animals, which has been harmonized with the existing EU legislation (Council Directive 86/609/EEC).

2.2. Induction of colitis and treatment protocol

C57BL/6 mice are a commonly used strain in studying DSS-induced ulcerative colitis (Yeganeh et al., 2018; Power et al., 2016; Detel et al., 2016; Yeom and Kim, 2015; Wagner et al., 2013). DSS is usually administered in a dose of 2–4% (w/v) dissolved in drinking water for 5–10 days. In the current study, colitis was induced by DSS (molecular mass 50 kDa; MP Biomedicals, Solon, OH, USA) at a concentration of 2.5% (w/v). Mice were randomly separated into four groups as follows: i) untreated mice (control group), ii) mice treated only with DSS (DSS group) and iii) DSS treated mice with supplementation of ChA 100 mg/kg and 200 mg/kg (ChA, Sigma-Aldrich, Steinheim, Germany). Mice were exposed to DSS solution from day 0 to day 7, with free access to the DSS solution, which was freshly prepared every other day. The mean DSS solution consumption was recorded for each group. The control group received tap water during the whole experimental period. ChA (CAS No. 327-97-9, > 95% purity, Merck, Darmstadt, Germany, a gift from Jadran Galenic Laboratory, Rijeka, Croatia), was dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich, Steinheim, Germany) and diluted in saline (5% DMSO solution) and given daily via oral administration (p.o.) by using the intragastric gavage technique. In order to evaluate potential therapeutic effect of ChA in DSS-induced colitis, mice received ChA at dose of 100 and 200 mg/kg, p. o. once a day from day 3 to day 7 and were sacrificed 4–6 h after receiving the last administration. ChA doses were selected according to our preliminary research as the most appropriate in order to provide good clinical manifestations with low animal mortality. Mice were anaesthetized by intraperitoneal administration of ketamine (2.5 mg/mouse) and sacrificed by cervical dislocation on day 7. Each experimental group consisted of 6–7 mice. At necroscopy, the entire colon was excised, after measuring the length of the colon it was rinsed with cold saline solution, cut into small pieces and fixed in 4% paraformaldehyde and embedded in paraffin, or snaps frozen in liquid nitrogen and stored at −80 °C for further analysis. Also, spleen was excised and its weight was measured to determine the spleen/body mass ratio.

2.3. Assessment of colitis severity

During the experimental period, the health status, changes in body weight and development of clinical symptoms (weight loss, severity of diarrhea, rectal bleeding) of colitis, were monitored daily. The disease activity index (DAI) was evaluated as described previously (Detel et al., 2016). Therefore, DAI was calculated as the average of scores for stool consistency, fecal bleeding and weight loss (DAI = (Stool consistency + Fecal bleeding + Weight loss)/3).

To evaluate histological damage paraffin-embedded colon segments were cut to 4 μm thick sections, deparaffinized, rehydrated with a series of xylene and aqueous alcohol solutions, respectively and stained with hematoxylin and eosin using standard procedures. The colon tissue sections were scored in a blinded fashion according to the previously published scoring criteria with few modifications (Shin et al., 2015; Cooper et al., 1993).

For histological examination, each colonic tissue specimen was graded based on the inflammation severity (none, 0; mild, 1; moderate, 2; severe, 3); the degree of inflammatory cell infiltration (normal, 0; mucosa, 1; mucosa plus submucosa, 2; transmural extension of the infiltrate, 3); the epithelial damage (intact, 0; crypt architecture distortion, 1; erosion, 2; ulceration, 3); the extent of lesions (none, 0; punctuate, 1; multifocal, 2; diffuse, 3); and edema score (none, 0; mild edema in the mucosa, 1; mucosa and submucosa, 2; entire wall of the colon, 3). The final histological damage score represents the sum of each individual score.

2.4. Immunohistochemical staining

2.4.1. For immunohistochemical staining colon tissue sections were

Deparaffinized and high-temperature antigen retrieval was performed by immersion of the slides in a 10 mmol/L trisodium citrate buffer, pH 6.0 for 20 min. After quenching of endogenous peroxidase with 3% hydrogen peroxide in methanol (v/v) for 30 min, the slides were washed with Tris-buffered saline (TBS, 0.01 M, pH 7.4) and incubated with 5% bovine serum albumin for 1 h. Expression of specific cell markers was determined by using primary antibodies against NF-κB p65 subunit (1:1000, ab7970).

Consequently, slices were incubated with primary antibodies in a humidified chamber at 4 °C overnight, while in a negative control the primary antibody was omitted. The staining was performed by using a DAKO EnVision™ + System, Peroxidase kit (Dako Denmark, Glostrup, Denmark) according to the manufacturer's instructions. 3,3′-diaminobenzidine chromogen was used as the substrate yielding a brown-colored precipitate. Counterstaining was performed with hematoxylin. Images from an Olympus BX40 (Olympus d. o.o., Zagreb, Croatia) microscope were captured and edited with a Pulnix TMC76S digital camera (PULNiX America Inc., Sunnyvale, CA, USA).

2.5. Western blot analysis

Colon tissue was homogenized in ice cold radioimmunoprecipitation assay lysis buffer with addition of 2 mM phenylmethyl sulphonyl fluoride, 1 mM sodium orthovanadate and 2 μg/ml of each aprotinin, leupeptin and pepstatin, for 20 min on ice (Potocnjak et al., 2017). After centrifugation at 15 000*g* for 20 min, the supernatant was collected and the protein content was analysed by the bicinchoninic acid (Thermo Scientific Pierce, Rockford, IL, USA) method. Equal