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Role of α-lipoic acid in dextran sulfate sodium-induced ulcerative colitis in mice: Studies on inflammation, oxidative stress, DNA damage and fibrosis

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ABSTRACT

Ulcerative colitis affects many people worldwide. Inflammation and oxidative stress play a vital role in its pathogenesis. Previously, we reported that ulcerative colitis leads to systemic genotoxicity in mice. The present study was aimed at elucidating the role of α-lipoic acid in ulcerative colitis-associated local and systemic damage in mice. Experimental colitis was induced using 3%w/v dextran sulfate sodium in drinking water for 2 cycles. α-Lipoic acid was administered in a co-treatment (20, 40, 80 mg/kg bw) and post-treatment (80 mg/kg bw) schedule. Various biochemical parameters, histological evaluation, comet and micronucleus assays, immunohistochemistry and western blot analysis were employed to evaluate the effect of α-lipoic acid in mice with ulcerative colitis. The protective effect of α-lipoic acid was mediated through the modulation of nuclear factor kappa B, cyclooxygenase-2, interleukin 17, signal transducer and activator of transcription 3, nuclear erythroid 2-related factor 2, NADPH: quinone oxidoreductase-1, matrix metalloproteinase-9 and connective tissue growth factor. Further, ulcerative colitis led to an increased gut permeability, plasma lipopolysaccharide level, systemic inflammation and genotoxicity in mice, which was reduced with α-lipoic acid treatment. The present study identifies the underlying mechanisms involved in α-lipoic acid-mediated protection against ulcerative colitis and the associated systemic damage in mice.

1. Introduction

Ulcerative colitis (UC) is a chronic gastrointestinal disorder that represents aberrant immune responses with adverse clinical outcomes (Poxton et al., 1997). UC results from an inappropriate and continuous inflammatory response to commensal microbes in a genetically susceptible host. In UC, inflammation is limited to colon, *i.e.*, it starts from the rectum and spreads proximally in a continuous fashion (Khor et al., 2011). It has been reported that UC is associated with an increased levels of various inflammatory markers such as myeloperoxidase (MPO), interleukin (IL)-1β, IL-6, IL-17, tumor necrosis factor-alpha (TNF-α), nuclear factor-kappa B (NF-κB) and cyclooxygenase-2 (COX-2) (Biesiada et al., 2012; He et al., 2012; Trivedi and Jena, 2012a; Zhao et al., 2013). IL-6 has been known to induce signal transducer and activator of transcription 3 (STAT3), and it also leads to an increased expression of IL-17 (Ivanov et al., 2006; Mudter and Neurath, 2007). Further, inflammation is associated with elevated oxidative stress, which in turn, is known to alter the expression of a redox sensitive transcription factor, nuclear erythroid 2-related factor 2 (Nrf2) (Kaspar et al., 2009). Nrf2 knockout mice have been known to be more susceptible to dextran sulfate sodium (DSS)-induced colitis and colitis-associated colorectal cancer suggesting the protective role of Nrf2 in colitis and the associated cancer (Khor et al., 2006, 2008). On the other hand, inflammation and oxidative stress lead to fibrosis and DNA damage in different organs (Cheresh et al., in press; Trivedi and Jena, 2012b). In UC, along with colon, other organs are also affected, and oxidative stress is mainly responsible

Abbreviations: UC, ulcerative colitis; MPO, myeloperoxidase; IL, interleukin; TNF-α, tumor necrosis factor-alpha; NF-κB, nuclear factor-kappa B; COX-2, cyclooxygenase-2; STAT3, signal transducer and activator of transcription 3; Nrf2, nuclear erythroid 2-related factor 2; DSS, dextran sulfate sodium; LPS, lipopolysaccharide; LA, α-lipoic acid; MMP-9, matrix metalloproteinase-9; VCAM-1, vascular cell adhesion protein-1; NQO-1, NADPH: quinone oxidoreductase-1; CTGF, connective tissue growth factor; End-III, endonuclease-III; FPG, formamidopyrimidine DNA glycosylase; H&E, hematoxylin and eosin; DMSO, dimethylsulphoxide; NMPA, normal melting point agarose; LMPA, low melting point agarose; EDTA, ethylenediamine tetraacetic acid; HBSS, Hank's balanced salt solution; DAI, disease activity index; MPO, myeloperoxidase; TBARS, thiobarbituric acid-reactive substances; GSH, reduced glutathione; TL, tail length; TM, tail moment; OTM, olive tail moment; % DNA, % DNA in comet tail; DAB, 3,3′-diaminobenzidinetetrahydrochloride.

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for UC-associated local as well as global damage (Jena et al., 2012; Trivedi and Jena, 2012a,b). Further, UC has been known to damage the colon mucosa and increase the gut permeability enabling the gut bacteria to translocate into other organs and cause tissue injury (Toedter et al., 2012; Trivedi and Jena, 2012b). Increased bacterial translocation is associated with elevated lipopolysacharide (LPS) level, which is pro-inflammatory in nature and is known to cause tissue damage in different organs (Cho et al., in press; Gabele et al., 2011; Trivedi and Jena, 2012b). As various molecular pathways are involved in the development and progression of UC, finding an appropriate agent to ameliorate the severity of the disease has become a challenging task.

α-Lipoic acid (1,2-dithiolane-3-pentanoic acid) (LA), isolated from bovine liver in 1950, is a naturally occurring sulfhydryl compound found in virtually all plant and animal species, and it is a potent anti-oxidant and anti-inflammatory agent (Odabasoglu et al., 2011a). It has become a common ingredient in multivitamin formulas and anti-aging supplements (Shay et al., 2009). It is readily taken up by a variety of cells and tissues and is reduced in mitochondria to dihydrolipoic acid (DHLA) (Packer et al., 1995). Both, LA and DHLA are amphipathic molecules and act as antioxidants both in hydrophilic and lipophilic environments (Moini et al., 2002). Moreover, LA and DHLA are involved in the regeneration of exogenous and endogenous antioxidants such as vitamin C, vitamin E and glutathione, chelation of metal ions and repair of oxidized proteins (Biewenga et al., 1997). LA is supposed to be synthesized in human and animal body in mitochondria and has been reported to reduce diabetic cardiomyopathy in rat through the suppression of mitochondrial oxidative stress (Bilska and Wlodek, 2005; Li et al., 2009). Apart from its anti-oxidant activity, LA is known to combat inflammation in various inflammatory disorders (Abdin et al., 2010; Bitar et al., 2010; Harding et al., 2012; Xu et al., 2012). It has been reported that LA inhibits adhesion molecule expression in human aortic endothelial cells and monocyte adhesion by inhibiting the NF-κB/IκB signaling pathway (Zhang and Frei, 2001). It has also been shown to activate phase II detoxification via the transcription factor Nrf2 and lower the expression of matrix metalloproteinase-9 (MMP-9) and vascular cell adhesion molecule-1 (VCAM-1) through repression of NF-κB (Shay et al., 2009). It has been described as a potent biological anti-oxidant, a detoxification agent, a modulator of various inflammatory signaling pathways and has been used to improve various disorders (Odabasoglu et al., 2011b; Scott et al., 1994; Smith et al., 2004; Suh et al., 2004). This impressive array of cellular and molecular targets of LA has raised considerable interest among the researchers in developing it as an interventional tool for many pathological conditions. Several clinical trials with LA show that it is advantageous for various disorders such as diabetic polyneuropathy, Alzheimer's disease, multiple sclerosis, hepatitis and obesity (Han et al., 2012; Koh et al., 2011; Maczurek et al., 2008; Melhem et al., 2005; Ziegler et al., 1999). However, to the best of our knowledge, only one preliminary study depicts the modulatory effect of LA against gut inflammation induced by trinitrobenzene sulfonic acid in rat (Kolgazi et al., 2007). Further, the detailed molecular mechanisms involved in LA-mediated protection against UC need to be explored both in experimental and clinical set-ups.

Previously, we reported that UC is associated with local as well as systemic damage in mice (Trivedi and Jena, 2012a). The present study was aimed at deciphering the mechanisms involved in the protection mediated by LA against UC-associated local as well as systemic damage in mice. LA has been reported to exert its beneficial effects in conditions where inflammation and oxidative stress play a vital role in the induction of cellular damage. Hence, LA was considered for the present study to elucidate the possible beneficial effect against DSS-induced UC in mice. The results of the present study indicate that LA reduced the severity of UC by decreasing inflammation, oxidative stress, fibrosis, DNA damage and cytotoxicity in the colon of mice through the modulation of various molecular targets such as NF-κB, COX-2, IL-17, STAT3, Nrf2, NADPH: quinone oxidoreductase-1 (NQO-1), MMP-9 and connective tissue growth factor (CTGF). Further, it reduced UC-induced elevated gut permeability and plasma LPS level, which was associated with a significant reduction in the systemic inflammation and genotoxicity.

2. Materials and methods

2.1. Animals

All the animal experiment protocols were approved by the Institutional Animal Ethics Committee (IAEC), and the experiments on animals were performed in accordance with the Committee for the Purpose of Control and Supervision of Experimentation on Animals (CPCSEA) guidelines. Experiments were performed on male Swiss mice (25–28 g) procured from the Central Animal Facility (CAF) of the institute. All the animals were kept under controlled environmental conditions at room temperature (22 ± 2 °C) with 50 ± 10% humidity and controlled cycle of 12 h light and 12 h dark. Standard laboratory animal feed (purchased from commercial supplier) and water were provided *ad libitum*. Animals were acclimatized to the experimental conditions for a period of 1 week prior to the commencement of the experiment.

2.2. Chemicals

DSS (MW 36,000-40,000 Da, CAS no. 9011-18-1) was purchased from MP Biomedicals. LA (CAS no. 1077-28-7), acridine orange (CAS no. 10127-02-3), endonuclease-III (End-III), formamidopyrimidine DNA glycosylase (FPG), SYBR Green I (CAS no. 163795-75-3), hexadecyltrimethylammonium bromide (CAS no. 57-09-0), o-dianisidine dihydrochloride (CAS no. 20325-40-0), 1,1,3,3-tetramethoxy propane (CAS no. 102-52-3), 2-thiobarbituric acid (CAS no. 504-17-6), bovine serum albumin (CAS no. 9048-46-8), hematoxylin and eosin (H & E) and trizma (CAS no. 77-86-1) were purchased from Sigma–Aldrich Chemicals, Saint Louis, MO, USA. Dimethylsulphoxide (DMSO), normal melting point agarose (NMPA), low melting point agarose (LMPA), triton X-100, ethylenediamine-tetraacetic acid (EDTA) and Hank's balanced salt solution (HBSS) were obtained from HiMedia Laboratories Ltd., Mumbai.

2.3. Study rationality and experimental design

In the present study, DSS was used to induce experimental colitis in mice due to its simplicity in experimental implementation and high level of reproducibility. Further, DSS-induced colitis model is characterized by chronic inflammation due to repeated administration of DSS and is associated with the disruption of the colonic mucosal architecture, and it mimics the features of clinical UC (Nishiyama et al., 2012; Sha et al., 2013). Based on the broad spectrum anti-oxidant, anti-inflammatory and cytoprotective action of LA, it can be assumed that LA may ameliorate the severity of UC. To date inadequate efforts have been made to elucidate the protective role of LA against UC. Hence, it was considered for the present study with an aim to decipher its possible protective role against UC-associated local and systemic damage. For the induction of UC, mice were administered 3%w/v DSS dissolved in drinking water for 7 days followed by 14 days of normal drinking water, again followed by 7 days of DSS-treated water, *i.e.*, 2 cycles of DSS treatment. LA was administered in 2 different dosing protocols, *i.e.*, co-treatment and post-treatment. In the co-treatment protocol, animals were randomized into 6 groups, each group consisting of 8 animals. Group 1 received normal drinking water for 28 days and served as control. Group 2 received 80 mg/kg bw/day, po LA for 28 days and served as LA control. Group 3 received DSS (3%w/v) from days 1–7 and 22–28. During the remission period (day 8–21), mice were administered normal drinking water. Groups 4, 5 and 6 received DSS (3%w/v) similarly as in group 3, additionally they were administered 20, 40 and 80 mg/kg bw/day, po LA respectively for 28 days. All the animals were sacrificed on the 29th day. In the post-treatment protocol, UC was induced in a similar manner as in the co-treatment protocol. After the induction of UC, in one group, LA (80 mg/kg bw) was administered for 14 days, and in another group, LA (80 mg/kg bw) was administered for 28 days in order to elucidate its possible protective role on established UC. Detailed experimental design is shown in Fig. 1. Disease activity indices (DAI) were calculated to assess the extent of colitis.

2.4. Estimation of inflammation by myeloperoxidase (MPO) assay

MPO activity was determined as an indicator of polymorphonuclear leucocyte accumulation as described (Rachmilewitz et al., 1989) with some modifications. The colon tissue was homogenized in ice cold 50 mmol/l potassium phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethylammonium bro-

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