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Original article

New insights on the modulatory roles of metformin or alpha-lipoic acid *versus* their combination in dextran sulfate sodium-induced chronic colitis in rats

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ABSTRACT

Background: Dextran sulfate sodium (DSS)-induced colitis is the most widely used model that resembles ulcerative colitis (UC) in human with challenging chronic mechanistic oxidative stress-inflammatory/immunological cascades. In models of acute colitis, reduction of oxidative stress and inflammatory burdens beside manipulation of many transcriptional factors were achieved by metformin or alpha-lipoic acid (α-LA). Currently, *in vivo* DSS-induced chronic colitis was conducted and the possible therapeutic roles of metformin and/or α-LA were explored.

Methods: Chronic UC was induced by adding 5% DSS orally in drinking water for 7 days followed by 3% DSS in drinking water for 14 days in adult male albino Wistar rats. Intraperitoneal administration of α-LA (25 mg/kg, twice/day) and/or metformin (100 mg/kg/day) were set at day 7 of DSS administration and continued for 14 days. Body weights, survival rates, disease activity index (DAI), colonic oxidative stress markers, tumor necrosis factor (TNF)-α levels, colonic nuclear factor-kappa-B (NF-κB) immunohistochemical expression, and the colonic histopathological changes were observed.

Results: Metformin or/and α-LA attenuated the severity of the DSS-induced colitis through improving the reductions in body weights, the DAI, the colonic oxidative stress markers, TNF-α, and NF-κB levels, and the morphological mucosal damage scores. Significant synergetic therapeutic effects were observed with combined therapeutic regimens.

Conclusion: Therapeutically, metformin and α-LA could be administered in chronic colitis. The combination of currently used pharmaceutics with natural and synthetic potent antioxidant compounds will become a therapeutic strategy of choice for UC to improve the quality of life if sufficient clinical trials are available.

Introduction

Inflammatory bowel disease (IBD), including ulcerative colitis (UC), is characterized by chronic relapsing intestinal inflammation. It has been a worldwide health-care problem with a continually increasing incidence among all ages [1–3].

Although the complexed pathophysiological events in IBD, the multifaceted interactions between the genetic/environmental/microbial factors and the triggered oxidative stress, and pro-/inflammatory burdens are emphasized, which is under the tight innate and adaptive immune regulations [4–7].

Regarding IBD with cronic courses, tumor necrosis factor-alpha (TNF-α), is an essential multifunctional cytokine. It is formed primarily by the reactive oxygen species-activated monocytes/macrophages and neutrophils recruitment, with subsequent crucial initiatory and continuous inflammatory/immunological roles [8–10], including the activation of signals of the transcriptional pathways [9,11].

In UC, the activation of nuclear factor-kappa B (NF-κB), a key transcriptional signaling pathway, is resulting in upregulation of multiple genes involved in the cellular production of pro-inflammatory cytokines as interleukin (IL)-6, IL-8 IL-1β, IL-10 and TNF-α, and subsequent inflammatory reactions [12,13]. Additionally, in the inflamed intestinal mucosa, NF-kB is a

Abbreviations: CAT, Catalase; DAI, Disease activity index; DSS, Dextran sulfate sodium; ELISA, Enzyme linked immunoassay; IBD, Inflammatory bowel disease; IL, Interleukin; α-LA, Alpha-lipoic acid; MDA, Malondialdehyde; NF-κB, Nuclear factor-kappa-B; PBS-BSA, Phosphate-buffered saline-bovine serum albumin; SOD, Superoxide dismutase; TNF-α, Tumor necrosis factor-alpha; UC, Ulcerative colitis.

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redox-sensitive transcription factor which is activated by oxidative stress [14–16].

Dextran sulfate sodium (DSS)-induced colitis is a reproducible model which morphologically likes UC in human [17,18]. It causes distortion in the epithelial integrity with increasing colonic mucosal permeability [19,20].

Metformin is one of the biguanides antidiabetic drugs, with approved multiple anti-oxidative, and anti-inflammatory properties against various inflammatory diseases [21–23]. In DSS-induced acute colitis and in cancer colon, the oxidative stress, inflammation, and apoptosis-associated NF-κB transcriptional activation are prohibited by metformin through decreasing the oxidative burden, and the pro-inflammatory mediators as TNF-α, and the stimulation of the 5′ adenosine monophosphate-activated protein kinase in the colon [21–25].

The α-lipoic acid (α-LA; 1,2-dithiolane-3-pentanoic acid, 6,8-dithio-octanoic acid, thioctic acid) is a naturally occurring antioxidant and a free radical scavenger, which is synthesized in small amounts in plants, animals, and humans and playing an important role as a cofactor in the metabolism [26,27].

In DSS-induced colitis, the defensive effects of α-LA have interceded via the restoration of the anti-oxidative milieu, the amelioration of the pro-inflammatory mediators as TNF-α, and interferon-gamma, and modification of many transcriptional factors including NF-κB [28,29].

In spite of widespread IBD conducted researches including UC, chronic nature of these diseases is considering an existing challenge. Additionally, the individual *versus* combined-therapeutic roles of metformin and α-LA on DSS-induced chronic colitis in rats have not been well explored yet. Therefore, the current work designed to study how would be the therapeutic potentials of α-LA and metformin each individually *versus* their combination on DSS-induced chronic colitis in rats.

Materials and methods

Animals

Sixty-four adult male albino Wistar rats, of 120–150 g, were used in this study. All rats were housed under normal light/dark cycle, at temperature of 25 °C ± 2, housed in plastic polyethylene cages (eight per cage) with free access to food and water, being maintained on a diet composed of (20% casein, 15% corn oil, 55% corn starch, 5% salt mixture, and 5% vitamins). Animals allowed for acclimatization for one week before the start of the study. All experimental dealings were permitted by the institutional animal care and use committee, which is following the National Institutes of Health guide for the care and use of laboratory animals (Maryland, USA).

Drugs and chemicals

The DSS (Affymetrix, USA) was purchased as a white crystalline powder (MW 40–50 kDa). Metformin and α-LA were purchased as a white powder (Sigma-Aldrich Company, St. Louis., USA). α-LA was dissolved in a physiological saline solution containing 0.5% NaOH, and the pH of the solution was adjusted to 7.4 with HCl and was administered by intraperitoneal injection at a dose of 25 mg/kg twice a day [28]. Metformin was dissolved in distilled water freshly and administered daily by oral gavage at a dose of 100 mg/kg/day [30].

Kits for malondialdehyde (MDA), superoxide dismutase (SOD), and catalase (CAT) were purchased from Biodiagnostic Company (Giza, Egypt), while TNF-α enzyme-linked immunoassay (ELISA) kits and polyclonal antibody kits for NF-κB immunohistochemistry were purchased from (Sigma-Aldrich Company, St. Louis., USA). Feces occult blood was determined using occult blood test device (ACON Laboratories, Cairo, Egypt).

Induction of chronic colitis

Chronic colitis was induced by adding 5% DSS orally in drinking water for 7 days followed by 3% DSS in drinking water for 14 days [20,31]. The day of DSS administration was defined as day (1).

Study groups

Rats were divided randomly into 8 groups of 8 animals each. Normal control group; rats without colitis, where no medications were given. α-LA or metformin control groups; rats without colitis received α-LA or metformin for 14 days respectively. α-LA and metformin control group; rats without colitis who co-administered freshly prepared α-LA and metformin for 14 days. DSS-induced chronic colitis group; where chronic colitis was induced by DSS in eight rats. α-LA-treated or metformin-treated groups; rats with colitis were given α-LA or metformin starting from day 7 of DSS and for 14 days respectively. α-LA and metformin-treated group; rats with colitis where α-LA and metformin were given together from day 7 of DSS administration and for 14 days.

Assessment of disease activity index (DAI)

Body weight of each animal was measured immediately before the experiment starting (day 1) and just before scarification. DAI assessment is the combined score of weight loss compared to initial weight, stools consistency, and bleeding [32]. Scores were defined as follows: weight loss: 0 (no loss), 1 (1–5%), 2 (5–10%), 3 (10–20%), and 4 (>20%); stools consistency: 0–1 (normal), 2–3 (loose stools), and 4 (diarrhea); and bleeding: 0 (no blood), 1 (Hemoccult positive), 2–3 (Hemoccult positive and visual pellet bleeding), and 4 (gross bleeding, blood around anus). The total clinical score was calculated by summation of the previous parameters. It was classified as a mild activity: 1–4, moderate activity: 5–8, and maximal activity: 9–12.

Macroscopic assessment of colon ulcers

At the end of the study, animals were sacrificed by cervical dislocation. The abdominal cavity was opened and the entire colon was removed and rinsed with saline. Specimens of the colon were opened, examined, scored and photographed for visible mucosal damage [33]. Colon mucosal damage was scored, using a 5-point scale, 0: no damage; 1: localized hyperemia, but no ulcer or erosions; 2: ulcer or erosions with insignificant inflammation; 3: ulcer or erosions with inflammation at one site; 4: two or more major sites of ulceration and/or inflammation; 5: two or more major sites of ulceration and inflammation extending more than 1 cm along the length of the colon [34].

Biochemical assessment

Colon tissue samples were weighed and 0.5 g samples were homogenized then were centrifuged for 15 min at 17,000 rpm. The supernatants were collected and kept frozen at −80 °C for subsequent biochemical studies.

Colon MDA assay

The extent of lipid peroxidation was determined as the concentration of thiobarbituric acid reactive substances (TBARS). The amount of MDA formed was measured spectrophotometrically at 532 nm as nmol per mg protein [35].