Food and Chemical Toxicology 47 (2009) 1311–1317

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Prophylactic role of curcumin in dextran sulfate sodium (DSS)-induced ulcerative colitis murine model

Hossam M.M. Arafa *, Ramadan A. Hemeida, Ali I.M. El-Bahrawy, Farid M.A. Hamada

Department of Pharmacology and Toxicology, Faculty of Pharmacy, Al-Azhar University, Nasr City, Cairo 11231, Egypt

ARTICLE INFO

Article history:
Received 24 November 2008
Accepted 4 March 2009

Keywords:
Curcumin
Dextran sulfate sodium (DSS)
Ulcerative colitis
TNF-α
NO
MPO
Anti-oxidant

ABSTRACT

We have addressed in this study the possible protective role of the main principle of turmeric pigment; curcumin on a murine model of ulcerative colitis (UC). Colitis was induced by administration of dextran sulfate sodium (DSS) (3% W/V) in drinking water to male Swiss albino rats for 5 consecutive days. DSS challenge induced UC model that was well characterized morphologically and biochemically. DSS produced shrinkage of colon length and increased the relative colon weight/length ratio accompanied by mucosal edema and bloody stool. Histologically, DSS produced submucosal erosions, ulceration, inflammatory cell infiltration and crypt abscess as well as epithelioglandular hyperplasia. The model was confirmed biochemically, and the test battery entailed elevated serum tumor necrosis factor (TNF-α) and colonic activity of myleoperoxidase (MPO). Colonic glutathione-*S*-transferase (GST) activity and its substrate concentration; GSH, were notably reduced, while lipid peroxidation, expressed as malondialdehyde (MDA) level, and total nitric oxide (NO) were significantly increased. Prior administration of curcumin (100 mg/kg, IP) for 7 consecutive days ahead of DSS challenge mitigated the injurious effects of DSS and ameliorated all the altered biochemical parameters. These results suggest that curcumin could possibly have a protective role in ulcerative colitis probably via regulation of oxidant/anti-oxidant balance and modulation of the release of some inflammatory endocoids, namely TNF-α and NO.

1. Introduction

Inflammatory bowel diseases (IBD) are chronic relapsing inflammatory disorders of the gastrointestinal tract (Geier et al., 2007). Being one of the IBD, ulcerative colitis (UC) is a disorder involving the large intestine and characterized by contiguous inflammation of the colonic lamina propria with subsequent injury and disruption of the mucosal barrier (Herias et al., 2005). The pathogenesis of the disease remains elusive and multifactorial. Its etiology remains unclear, but it appears to result from a dysregulated immune response, with infiltration of phagocytic leukocytes into the mucosal interstitium. The production and release of reactive oxygen species by immune cells seem to play a crucial role in the physiopathology of UC (Damiani et al., 2007). Oxidative stress with its dual of free radical generation and enhanced lipid peroxidation is the mainstay of disease evolution (Rezaie et al., 2007). Also, proinflammatory cytokines, to which TNF-α belongs, were found to play an important role in the progression of mucosal inflammation (Camacho-Barquero et al., 2007). Most of the treatment modalities for UC comprise anti-inflammatory therapy (5-aminosalicylic acid and glucocorticosteroids), immunomodulatory agents (azathioprine, mercaptopurines and cyclosporine) and monoclonal antibody that specifically target TNF-α (Kozuch and Hanauer, 2008). Many experimental surrogate models for UC have been developed in an attempt to exploit the exact events that interplay in such an organ malady. Dextran sodium sulfate (DSS) colitis paradigm is the most appropriate model that resembles, in many facets, the human phenotype (Kawada et al., 2007).

Curcumin is the main principal of the turmeric pigment of *Curcuma longa*. Curcumin has many beneficial effects including anti-inflammatory (Jacob et al., 2007), antioxidant (Kumar et al., 2007), anticarcinogenic (Thangapazham et al., 2008), antimicrobial (Goel et al., 2008), hepatoprotective (Farombi et al., 2008), anti-hyperlipidemic (Arafa, 2005) and anti-angiogenic (Maheshwari et al., 2006) actions. These pleiotropic effects made curcumin a suitable candidate to be incorporated in this study. The main objective of this work was to address whether or not curcumin could have a protective role in DSS-induced ulcerative colitis in male Swiss albino rats, and the mechanism(s) whereby the turmeric pigment would confer such protection, if any.

The biochemical test battery encompassed assessment of serum TNF-α level, colonic activities of MPO and GST as well as the colon contents of total NO, GSH, and MDA. Gross examination of colon length and weight and histological examination of colon sections with routine hematoxylin and eosin double stain were done to confirm the model and unravel any possible protection exerted by curcumin.

* Corresponding author. Tel.: +20 2 4577202; fax: +20 2 2014052.
E-mail address: hmmarafa@yahoo.com (H.M.M. Arafa).

0278-6915/$ - see front matter © 2009 Published by Elsevier Ltd.
doi:10.1016/j.fct.2009.03.003

2. Materials and methods

2.1. Drugs and chemicals

Curcumin and DSS were obtained from Fluka Chemical Co. (GmbH, Steinheim, Germany). Dimethoxybenzidine (DMB), dimethyl sulfoxide (DMSO), thiobarbituric acid, *n*-butanol, vanadium III chloride, 1-chloro-2,4-dinitrobenzene, Ellman's reagent (5,5′-dithio-bis-(2-nitrobenzoic acid), GSH, 1,1′,3,3′-tetramethoxypropane, hexadecyltrimethyl ammonium bromide were obtained from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals were of the finest analytical grade.

2.2. Animals

Male Swiss albino rats weighing 150–200 g were obtained from Theodor Bilharz Institute (Giza, Egypt). The animals were housed in the animal facility of the Faculty of Pharmacy, Al-Azhar University. The animals were fed a standard diet (El-Nasr Company, Abou-Zaabal, Cairo, Egypt), and allowed free access to water. The rats were kept under standard conditions of temperature (21 ± 0.5 °C) and relative humidity (55 ± 5) with 12-h light/12-h dark cycle. All animal experiments were conducted according to the regulations of the Committee on Bioethics for Animal Experiments of Al-Azhar University.

2.3. Experimental design

A total of 48 rats were allotted in four groups, 12 rats each. First group was given DMSO (50% v/v) (0.2 ml/100 g, IP) once daily for 7 consecutive days and served as control group. Second group was administered curcumin (100 mg/kg, IP) once daily for 7 consecutive days. Third group was challenged with DSS (3% w/v) orally in drinking water for 5 consecutive days as previously described by Tsune et al. (2003). Fourth group received curcumin as a single dose (100 mg/kg, IP) as before and kept thereafter on DSS (3% w/v) in the same dose regimen. Twenty-four hours after the DSS regimen, animals were anesthetized with light ether, and retroorbital blood samples were withdrawn using heparinized microcapillaries (Optilab, Berlin, Germany). Serum was separated following centrifugation at 4000 rpm for 10 min at 4 °C. Animals were then euthanized by cervical dislocation. Colons were dissected out, plotted blotted dry on filter paper and homogenized in ice-cold 0.15 M KCl using Elvehjem glass potter (Berlin, Germany) to give a final concentration of 20% homogenate. Two colons from each group were kept in 10% formol saline prior to hisopathological examination.

2.4. Methods

2.4.1. Assessment of serum TNF-α level

RaTNF-α was determined in serum using a raTNF-α sandwich ELISA kit (Biosource International, CA, USA). The method utilizes the immunoassay technique previously described by Wolters et al. (1977). The assay simply involves a four-member sandwich reaction in which streptavidin-peroxidase enzyme acts on a specific substrate, releasing a colored product measured at 450 nm. Color intensity is directly proportional to the concentration of raTNF-α. The cytokine concentration was computed from a calibration curve of raTNF-α.

2.4.2. Determination of colonic MPO activity

MPO activity was assessed according to Manktelow and Meyer (1986). Colonic MPO was extracted with hexadecyltrimethyl ammonium bromide. Then, dimethoxybenzidine (DMB) was oxidized by MPO in presence of hydrogen peroxide, and the optical density was measured at 460 nm. Optical density was a direct measure of enzymatic activity.

2.4.3. Determination of colonic total NO content

Total nitric oxide (NO) was determined by measuring its stable metabolites in particular nitrite (NO_2^-) and nitrate (NO_3^-) according to the method described by Miranda et al. (2001). In brief, 0.1 ml of colonic homogenate (20%) was added to 0.1 ml of methyl alcohol and then centrifuged at 3000 rpm for 10 min. An aliquot of the resultant supernatant (0.1 ml) was aspirated and mixed with 0.1 ml of vanadium (III) chloride. Then, 50 µl of sulfanilamide solution and 50 µl of *N*-(1-naphthyl) ethylene diamine dihydrochloride (NEDD) were added respectively, and incubated at 37 °C for 30 min. The optical density was measured at 540 nm against a blank using Shimadzu spectrophotometer UV, 1201(Japan).

2.4.4. Determination of reduced glutathione (GSH)

GSH was estimated following the earlier method of Ellman (1959). Colonic homogenate (0.5 ml) was mixed with 0.5 ml of 10% trichloroacetic acid. The contents were mixed well for complete precipitation of proteins and centrifuged at 2000 rpm for 5 min. An aliquot of clear supernatant (0.1 ml) was taken and mixed with 1.7 ml of 0.1 M potassium phosphate buffer (pH 8). Ellman's reagent (0.1 ml) was added. After 5 min, the optical density was measured at 412 nm against blank.

2.4.5. Determination of glutathione-S-transferase (GST) activity

The GST activity towards 1-chloro-2,4-dinitrobenzene in presence of glutathione as a co-substrate was measured spectrophotometrically at 25 °C according to the method described by Habig et al. (1974). The enzyme activity was determined by monitoring the change in absorbance at 340 nm.

2.4.6. Determination of lipid peroxidation

Lipid peroxidation was assessed as MDA content of the colon according to the method of Uchiyama and Mihara (1978). In short, the colorimetric determination of MDA is based on the reaction of one molecule of the reactive aldehyde with two molecules of thiobarbituric acid at low pH (2–3), and at a temperature of 95 °C for 45 min. The resultant pink color was extracted by *n*-butanol, and the absorbance was determined at 535 and 520 nm spectrophotometrically. The difference in optical density between both wavelengths was used as a measure of colonic MDA content.

2.4.7. Colonic length and relative colonic weight/length ratio (g/cm)

After animals were killed by cervical dislocation and the entire colon was removed, gently flushed with saline, placed on an ice-cold plate, cleaned of fat and mesentery, and blotted on filter paper to dry. Each colon was weighed and its length was measured.

2.4.8. Histopathological examination

Colons were kept in 10% formalin solution for 24 h using Hartz Technique (1947). Tissues were then embedded in paraffin blocks, and 5-µm-thick sections were obtained from the blocks and stained by hematoxylin and eosin and examined microscopically. Examination was done blind by the histologist.

2.4.9. Statistical analysis

Data analysis was achieved using a software program GraphPaD InStat (version 2.0, Philadelphia, 1993). Data were presented as means ± SE. Comparisons were done using one way ANOVA followed by Tukey–Kramer as a post-ANOVA test. Criterion for significance was chosen to be at $p < 0.05$.

3. Results

3.1. Serum TNF-α Levels

Curcumin had no effect on serum TNF-α level compared to control group (Fig. 1). Challenge with DSS caused an apparent elevation in the level of serum TNF-α by about 69% compared to control animals. Pretreatment with curcumin before DSS administration resulted in a notable decrease in the concentration of serum TNF-α amounted to about 46% compared to rats treated with DSS alone (Fig. 1).

3.2. Myeloperoxidase (MPO) activity

Curcumin showed no effect on the colonic MPO activity compared to control rats (Fig. 2). The colonic MPO activity was greatly increased by about 150% following administration of DSS

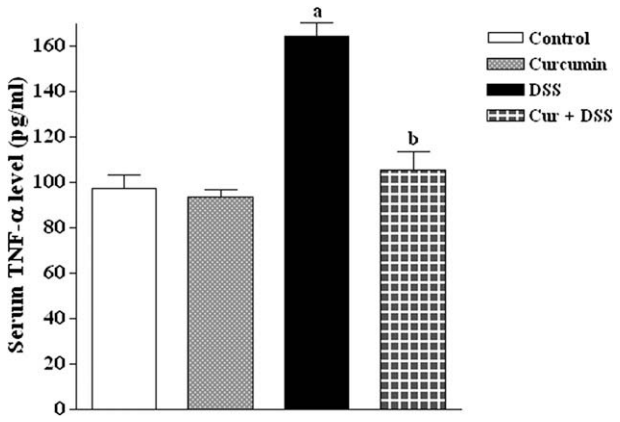

Fig. 1. Effects of curcumin and/or DSS on serum TNF-α levels in male Swiss albino rats (Data were presented as means ± SE, $n = 10$).