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RESEARCH ARTICLE

In vivo effects of curcumin on the paraoxonase, carbonic anhydrase, glucose-6-phosphate dehydrogenase and β-glucosidase enzyme activities in dextran sulphate sodium-induced ulcerative colitis mice

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Abstract

Increases in the risk of infections and malignancy due to immune suppressive therapies of inflammatory bowel diseases (IBDs) have led the researchers to focus on more nontoxic and acceptable natural products like curcumin. Here we investigate whether prophylactic and therapeutic application of the curcumin alters the enzyme activities of paraoxonase (PON), carbonic anhydrase (CA), glucose-6-phosphate dehydrogenase (G6PD) and cytosolic β-glucosidase in dextran sulphate sodium (DSS)-induced ulcerative colitis mice. Prophylactic application of curcumin resulted in higher MPO activity, less body weight loss and longer colon lengths compared to therapeutic group indicating preventive role of curcumin in IBDs. DSS-induced decrease in liver and serum PON activities were completely recovered by prophylactic administration of curcumin. DSS-induced reduction in liver cytosolic β-glucosidase activity was not affected by curcumin neither in the prophylactic group nor in the therapeutic group. Erythrocyte CA activity was significantly increased in curcumin groups, however no remarkable change in G6PD activity was observed.

Keywords

Curcumin, enzyme, inflammatory bowel disease, ulcerative colitis

History

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Introduction

Dietary habits related to lifestyle are important factors influencing human diseases and metabolism. Every chemical step of the metabolism is catalyzed by an enzyme and these biochemical reactions are negatively affected in metabolic diseases. Inflammatory bowel diseases (IBDs) including Crohn's disease and ulcerative colitis (UC) are chronic idiopathic inflammatory disorders characterized by chronic inflammation of the gastrointestinal tract with the presence of ulcers in the mucosa and altered bowel habits[1,2]. Etiology of IBD is still unknown, but dissecting the knowledge on the IBDs suggests that immune, genetic and environmental factors affect both the initiation and progression of IBDs[3]. IBD is generally treated using 5-amninosalicylates (5-ASA), antibiotics, the steroids and immune modulators in the clinic. Even using anti-inflammatory and/or immunosuppressive agents for the therapy of IBDs is effective, intolerabilities and adverse effects of agents are the main limiting factors of the therapy. As IBDs are chronic relapsing and remitting diseases and as the curative therapies for IBDs are currently unknown, regardless of immunosuppressive and biologic therapies that have potentially increased risk of infections and malignancy, there is an increasing interest for natural therapies in the treatment of IBD[1]. In fact, the pharma foods are gaining wide range of popularity over the globe. Studies specifically clinical trials have agreed that curcumin is a potential therapeutic agent for IBD[4].

Curcumin, a yellow pigment in the spice turmeric (*Curcuma longa*), has been used for centuries in herbal medicines for the treatment of a variety of ailments such as inflammatory diseases, diabetic ulcers, anorexia, cough and sinusitis[5]. The studies are showing that curcumin is the nontoxic and acceptable agent that is effective in preventing or ameliorating IBDs[6]. In addition to antibacterial, antifungal, antiviral and anti-proliferative activity, numerous studies have also been shown that curcumin possesses significant antioxidant and anti-inflammatory effects. Despite all these promising results, low bioavailability is reducing the efficacy of curcumin and seriously restricts its usage as a drug[7]. Phase I clinical trials have shown that curcumin is well tolerated at doses up to 12 g/day without clinically significant side effects but exhibits low bioavailability[7,8]. Poor absorption, rapid metabolism and elimination seem to be the main reasons contributing to the low bioavailability, resulting in low plasma and tissue levels of curcumin. Biodistribution of curcumin in body tissues is another important factor affecting its activity and administration of higher doses of curcumin does not result in higher absorption and higher uptake[7,9].

The role of oxidative stress in human diseases including chronic inflammatory disorders, alterations in the activity of the antioxidant and/or other enzymes in the organisms becomes more imperative research area for IBDs. Both exogenous and endogenous antioxidants have crucial role in controlling intestinal

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inflammatory stress. Experimental and clinical studies demonstrated an increased formation of reactive oxygen/nitrogen species (ROS/RNS) and also biomarkers of oxidative injury in colonic mucosa in the animal models of IBD and in intestinal mucosa of IBD patients[10–15]. Genetic variations in paraoxonase (PON) gene family that have antioxidant and anti-atherogenic properties in the regulation of cellular oxidative stress, is associated with altered enzyme activity and risk of developing IBD[10,16]. Furthermore, it was found that PON1 activity was inhibited during the acute-phase response showing that PON1 may have a function in the intestine as detoxifiers and antioxidants[17]. Although it is well known that low serum PON1 activity is significantly associated with a risk of atherosclerosis and associated with several diseases, little is known regarding the effect of serum and liver PON activity on IBDs[18]. Curcumin has strong antioxidant and anti-inflammatory activities and significant effort has been taken to evaluate the effects of curcumin in IBDs, however the effect of curcumin on serum and liver PON activities in UC mice and/or IBD patients is unknown. Carbonic anhydrase (CA) is another important antioxidant enzyme involved in the regulation of colonic electrolyte transport and pH by catalyzing the reversible hydration/dehydration of CO_2 and water. *In vivo* and *in vitro* studies demonstrate alterations in transmucosal ion exchange and metabolic imbalance of colonic mucosa indicating the importance of CA in IBDs[19–22]. Glucose-6-phosphate dehydrogenase (G6PD), an essential antioxidant, is the only enzyme in erythrocytes to counterbalance oxidative stress via regeneration of NADPH[23]. G6PD catalyses the conversion of glucose 6-phosphate to 6-phosphoglucono-δ-lactone with reduction of $NADP^+$ to NADPH and NADPH serves as hydrogen and electron donor for a variety of reductive reactions. G6PD deficiency causes worse inflammatory response during disease progression in IBDs and is found to be new promising target for cancer therapy as it is involved in apoptosis and angiogenesis[24–26]. β-Glucosidases play key roles in a variety of essential physiological processes as the β-glucosidase activity is important representative indices for the activity of probiotics related to IBDs[27–30]. In mammals, several β-glucosidases have been characterized, such as lysosomal β-glucosidase (acid β-glucosidase), lactase phlorizin hydrolase (LPH) and cytosolic β-glucosidase. Cytosolic β-glucosidase, present in the kidney, liver, spleen, intestine and lymphocytes, has important activity towards many common dietary xenobiotics, however there is limited information about the effect of curcumin on liver cytosolic β-glucosidase in mammals[31–35].

Concerning several reports related to the effect of curcumin on IBDs and considering the extensive usage of curcumin on food flavoring, we wondered the prophylactic and therapeutic effects of curcumin on the different metabolic enzymes in dextran sulphate sodium (DSS)-induced colitis mice. DSS-induced colitis model is the widely used classical disease model to study the mechanism of human IBDs, as this model results in stable occurrence of disease very similar to human IBDs[2,10,36–38]. The current work, therefore, extends to the determination of the following enzyme activities of DSS-induced colitis mouse; PON activity from serum and liver, CA and G6PD activity from erythrocytes, and cytosolic β-glucosidase activity from liver. The findings of this study are important to evaluate the curative or preventive mechanisms of curcumin to improve new strategies for the treatment of IBDs, as natural therapy is a hot research topic in the inflammatory diseases.

Methods

Animals and study design

The study was conducted on 6–7 weeks of age, female Balb/C mice that were purchased from Uludag University, Bursa, Turkey. The protocol of the study was approved by the local Ethics Committee of Uludag University (2013-05/05). The animals were fed a standard diet and had free access to drinking water or 5% dextran sodium sulfate polymers (DSS, MW 36–50 kDa, MP Biomedicals, OH) added with drinking water. They were maintained on a 12 h light/dark cycle at 21 °C. Curcumin and sulfasalazine were dissolved in olive oil and administered 100 mg/kg per mouse. Two main groups were addressed: the therapeutic group and the prophylactic group.

The therapeutic groups were formed to determine the therapeutic effects of curcumin administration in DSS-induced colitis mice. For the therapeutic groups, the mice were randomly divided into four groups, seven mice per group. Mice in control group consumed drinking water and administered only olive oil by oral gavage for seven days. DSS-induced colitis was generated in the mice in DSS group as described before[39–41]. Briefly, 5% DSS was added to the drinking water for seven days. Olive oil was also administered by oral gavage for these seven days. Last two groups were treatment groups. Mice in DSS + Curcumin group and DSS + sulfasalazine group were also exposed to 5% DSS in drinking water for seven days to induce colitis, and administered curcumin or sulfasalazine dissolved in olive oil by oral gavage, respectively, for the treatment of colitis (Figure 1). Sulfasalazine is the most frequently used drug with the best clinical outcomes in humans with IBD. It also has similar therapeutic effects on the treatment of DSS-induced colitis in Balb/C mice as it has in IBD in human[42]. In this study, DSS + sulfasalazine groups were

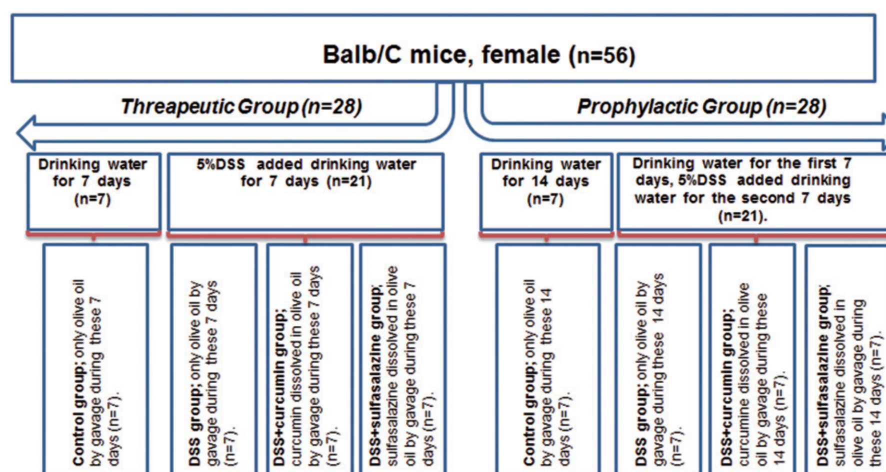

Figure 1. Experimental design of animal study.

included in order to compare the effects of curcumin with the known curative effects of sulfasalazine.

The prophylactic groups were formed to investigate the prophylactic effects of curcumin on occurrence of acute colitis attacks. The experiments of prophylactic groups were designed as described before[39,43]. Briefly, seven mice in the each of the control group consumed drinking water for 14 days and meanwhile administered only olive oil by oral gavage. Mice in DSS, DSS + Curcumin, DSS + sulfasalazine groups consumed drinking water for the first seven days and they consumed 5% DSS containing drinking water for the following seven days to induce colitis. During these 14 days, curcumin and sulfasalazine administrations were given to the DSS + curcumin and DSS + sulfasalazine groups using same dosage and application way as it was done in corresponding therapeutic groups.

At the end of the experiments, animals were anesthetized with ketamine (100 mg/kg) and xylazine (12 mg/kg). Anesthetized animals were euthanized by exsanguination. Blood samples were collected from the left ventricle of the heart and used for the determination of enzyme activities. Liver and colon samples were also removed and prepared for the enzyme assay as described below.

Histopathological methods

At the end of experimental procedures all animals were euthanized as described above. About 2 cm long colon segments from distal end of the colons were removed for histopathological examinations. Colon tissues were fixed with 10% neutral buffered formalin for 24 h. After the fixation, they were washed under running tap water to remove excess fixative. Fixed colon segments were dehydrated with graded alcohols, cleared with xylene and embedded in paraffin. Five-micrometer thick sections were cut from tissue blocks. All sections were stained with hematoxylin and eosin[44] and examined to evaluate the microscopic structure of the colon. An Olympus CX21 microscope was used for the examination of stained slides and pictures were taken with an Olympus SC100 camera.

Preparation of colon, liver and blood samples

Liver samples were used for the determination of the cytosolic β-glucosidase and PON activity. Briefly, livers were powdered with liquid nitrogen and then were separated into two parts. First part was placed in 2 volumes of ice-cold extraction buffer (0.1 M Tris–HCl pH 7.0) and was used for β-glucosidase assay. Second part was placed in 4 volumes of ice-cold homogenization buffer, nuclei and mitochondria were removed by successive centrifugation at 460 *g* for 10 min. The post-mitochondrial supernatant fraction was then centrifuged at 10 000 *g* for 60 min. The microsomal pellet was suspended in 20 ml of 5 mM Tris–HCl buffer, pH 7.4. The microsomal fraction was adjusted to 0.75% Triton X-100, vortexed, stored at 4 °C for 30 min and then centrifuged at 14 000 *g* for 60 min. The resultant supernatant fraction was used for PON enzyme assay[45,46].

G6PD, CA and serum PON enzyme activities were determined from blood. Blood samples were taken to dry tubes and centrifuged at 1500 rpm for 15 min. 0.5 ml serum was removed and used for PON enzyme assay. The packed red cells were hemolyzed with cold water. Hemolysate was used for G6PD and CA enzyme assays[47].

Colonic samples were used for MPO activity and powdered using liquid nitrogen. After homogenization in 1:20 (w/v) of 50 mM phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethyl ammonium bromide, samples were centrifuged at 14 000 rpm for 15 min and the supernatant was used for enzyme assay[48,49].

Enzyme assays

Cytosolic β-glucosidase activity was routinely determined using para-nitrophenyl-β-D-glucopyranosides (*p*-NPG) as substrates. Appropriately 70 μl of enzyme solution in 50 mM sodium acetate, pH 5.5 and 70 μl of substrate were mixed in the wells of a 96-well microtiter plate in triplicate. The reaction was stopped by adding 70 μl of 0.5 M Na_2CO_3, after incubation at 37 °C for 30 min and the color product was measured at 410 nm. Enzyme activity was expressed as μmol *p*-nitrophenol formed per minute in the reaction mixture under these assay conditions.

Serum and liver PON enzyme activity towards paraoxon was quantified spectrophotometrically by the method described by Gan et al.[50]. PON activity was quantified spectrophotometrically using 100 mM Tris–HCl buffer, pH 8.0 containing 2 mM $CaCl_2$. Reaction was initiated by the addition of 50 μl of serum or 100 μl of microsomal fraction and was followed for 2 min at 25 °C by monitoring the appearance of p-nitrophenol at 412 nm in spectrophotometer. All rates were determined in duplicate and corrected for the non-enzymatic hydrolysis. The final substrate concentrations during enzyme assay were 2 mM and 1.5 mM for microsomal fraction and serum, respectively.

The CA enzyme activity was assayed by following the hydration of CO_2 according to the method described by Wilbur and Anderson[51]. CO_2-hydratase activity of enzyme was determined at room temperature in a veronal buffer (pH 8.6) with phenol-red as an indicator and saturated carbon dioxide solution as a substrate in a final volume of 4.2 ml. The time (in seconds) taken for the solution to change from red to yellow was measured. The enzyme unit (EU) was calculated using the equation ($t_0 - t_c/t_c$) where t_0 and t_c are the times for the pH change of the non-enzymatic and enzymatic reactions, respectively.

G6PD activity assays were run at 25 °C according to Beutler's methods[52], which depended on the reduction of $NADP^+$ by G6PD, in the presence of glucose 6-phosphate. For the spectrophotometric measurements, the reaction mixture contained 10 mM $MgCl_2$, 0.2 mM $NADP^+$, and 0.6 mM G6P in 100 mM Tris–HCl buffer, pH 7.5, and 50 μl of the enzyme. The conversion of $NADP^+$ to NADPH was followed by monitoring the change in absorbance at 340 nm. One EU was defined as the enzyme amount reducing 1 μmol $NADP^+$ per min.

Neutrophil infiltration into colon was quantified by measuring MPO activity[53]. Briefly, supernatant of homogenized samples was added to 1 mg/ml o-dianisidine hydrochloride and 0.0005% hydrogen peroxide. Changes in absorbance at 460 nm were measured. One unit of MPO activity was defined as the amount that degraded 1 mmol peroxidase per minute at 25 °C.

THERMO microplate reader was used for the measurement of all enzyme assays except CA enzyme assay. Assays were carried out in duplicates.

Results

Main macroscopic parameters to represent disease in DSS-induced colitis model are the loss of body weight, shortening of the colon and rectal bleeding[54]. In our study, DSS administration (DSS; DSS + curcumin; DSS + sulfasalazine groups) showed mild rectal bleeding and loss of body weight in both the treatment and prophylactic group of animals (Figure 2A). In addition to the loss of body weight and rectal bleeding, colon lengths of the animals in the DSS treated groups were found to be significantly shorter than the control groups (Figure 2B). DSS-induced colitis is also characterized by histological haematoxylin and eosin staining (H&E). H&E stained sections of animals drinking DSS added water was compared with the sections of control group animals. In control group, normal colon morphology was seen (Figure 2C.a). But in DSS group (in therapeutic group), loss of epithelia and

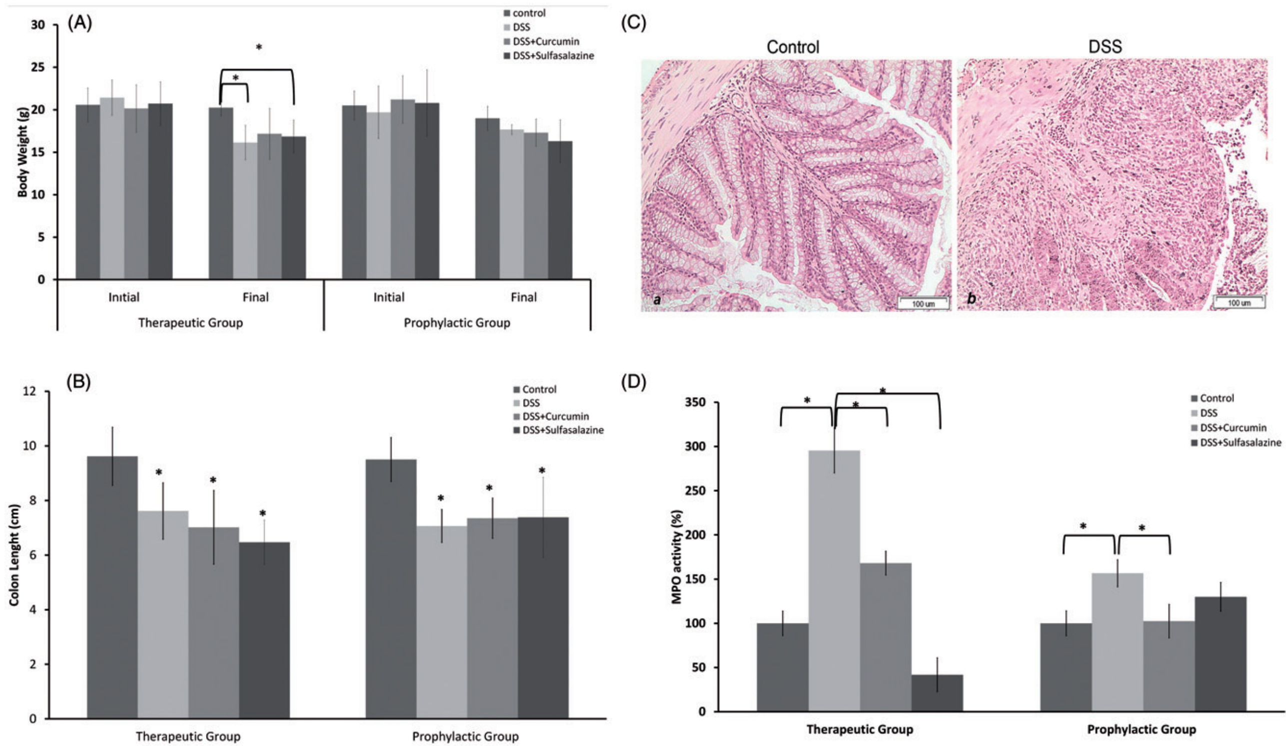

Figure 2. Effects of DSS, DSS + curcumin and DSS + sulfasalazine on the inflammatory parameters of DSS-induced colitis for prophylactic and therapeutic application. (A) Changes in the body weight, the weight of each mouse was taken in the first day of experiment (initial) and at the end of the experiment (final). (B) Changes in the colon lengths, (C) Histological findings, light micrographs of hematoxylin and eosin staining of mice in control group (a) and mice in DSS treated group and (b) for the prophylactic application. (D) Myeloperoxidase activity from colonic samples. Colonic samples were powdered using liquid nitrogen. After homogenization samples were centrifuged at 14 000 rpm for 15 min and supernatant was used for enzyme assay. The data represent means ± SD ($n = 7$ mice/group). *$p < 0.05$.

intestinal crypts, and thickening of both mucosa and sub-mucosa were detected. There was also pronounced inflammatory cell invasion in these layers of the organ wall. More inflammatory cells were also seen in muscular layer and in the lumen of the colon (Figure 2C.b). Colonic MPO activity is also used as marker for colonic injury related to neutrophil infiltration within the mucosa. As shown in Figure 2D, myeloperoxidase activity was significantly elevated in DSS-treated mice for both therapeutic and prophylactic group ($p < 0.05$).

The results of *in vivo* effects of DSS, DSS + curcumin and DSS + sulfasalazine on serum and liver PON activity are presented in Figure 3, DSS treatment inhibited both the serum and liver PON activity statistically significant compared to control group. However curcumin treatment increased the liver and serum PON activity, only increase in the prophylactic group was found to be significant. Sulfasalazine treatment did not affect the liver PON activity compared to DSS group but resulted in increase of serum PON activity. The results of CA and hepatic cytosolic β-glucosidase activity for therapeutic group (Figure 4A) and prophylactic group (Figure 4B) are shown in Figure 4. Erythrocyte CA activity was slightly affected by DSS and sulfasalazine treatment, as well as curcumin caused significant increase in CA activity compared to control group for both therapeutic and prophylactic groups. The hepatic cytosolic β-glucosidase activity was significantly inhibited in all groups compared to control group. Sulfasalazine treatment significantly elevated the cytosolic β-glucosidase activity compared to DSS group. Changes in the activity of G6PD were found to be quite contradictory for the therapeutic and prophylactic groups (Figure 5). While erythrocyte G6PD activity of curcumin treated group was found to be less than the control of prophylactic group, for the therapeutic group no changes were obtained between the G6PD activity of the control and curcumin group. Any of the variation in G6PD activity was not found to be statistically significant.

Discussion

IBDs are complex and multifactorial diseases associated with chronic relapsing inflammation of the intestinal tract with unknown etiology. The use of natural anti-inflammatory products is the focus of interest to prevent or control the inflammatory disorders and their nontoxic and tolerable properties[55]. In this work, we compared for the first time, the prophylactic and therapeutic effects of curcumin in DSS-induced colitis mice. Our results revealed that prophylactic administration of curcumin has led to more impressive results compared to therapeutic administration as prophylactic group has significantly higher serum and liver PON activity (Figure 3). In parallel to PON activity results, loss of body weight in prophylactic group was not found to be significant (Figure 2A) and MPO activity of prophylactic group was found lower than the therapeutic group (Figure 2D). Colon lengths of the DSS + curcumin and DSS + sulfasalazine groups in prophylactic group were also found longer than the colon lengths of counterparts in therapeutic group (Figure 2B).

DSS-induced inflammation was assessed by macroscopical findings, histopathological evaluation of the colon and measurement of colonic myeloperoxidase activity[3]. As there are several chemical methods to induce IBD in mice, DSS-induced colitis is the most prominent model because this model resulted in many similarities with human UC[54]. Consistent with the several reports on MPO activity of colonic samples from mice with IBD, our results also showed that IBD induction significantly increased the MPO activity compared to the control group (Figure 2D)[56,57]. Suppressor effect of curcumin on the development of DSS-induced colitis in mice was already demonstrated by

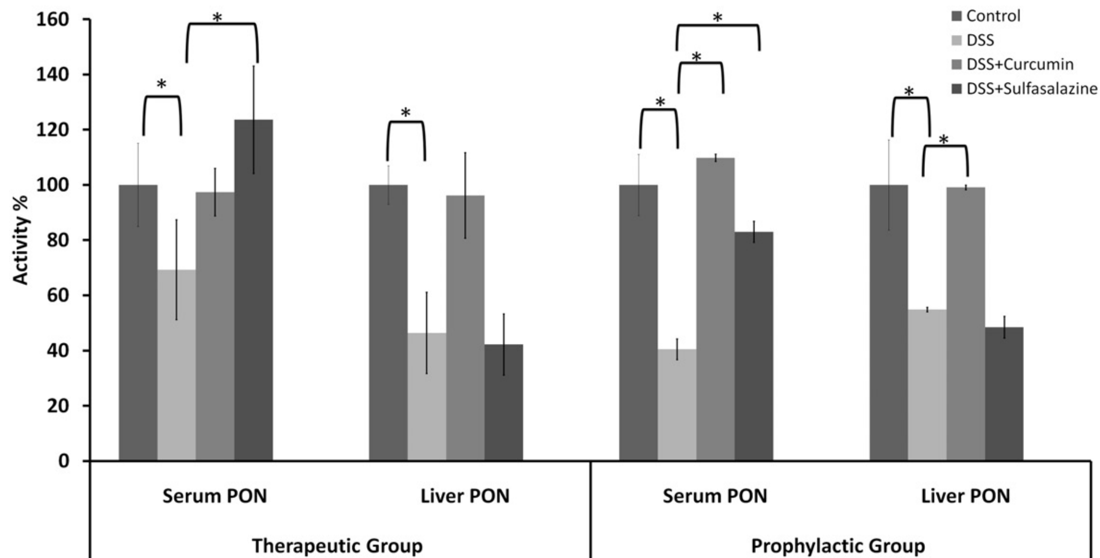

Figure 3. *In vivo* effects of DSS, DSS + curcumin and DSS + sulfasalazine on serum and liver paraoxonase activity of DSS-induced colitis mice for prophylactic and therapeutic application. Blood samples were collected in dry tubes and serum was separated by centrifugation. 0.5 ml serum was used for paraoxonase enzyme assay. Liver samples were powdered with liquid nitrogen and placed in 4 vol. of ice-cold homogenization buffer, centrifuged at 460 *g* for 10 min. Supernatant was then centrifuged at 10 000 *g* for 60 min, microsomal pellet was re-suspended and centrifuged at 14 000 *g* for 60 min. The resultant supernatant fraction was used for paraoxonase enzyme assay. The data represent means ± SD ($n = 7$ mice/group). All rates were determined at least in duplicate. *$p < 0.05$.

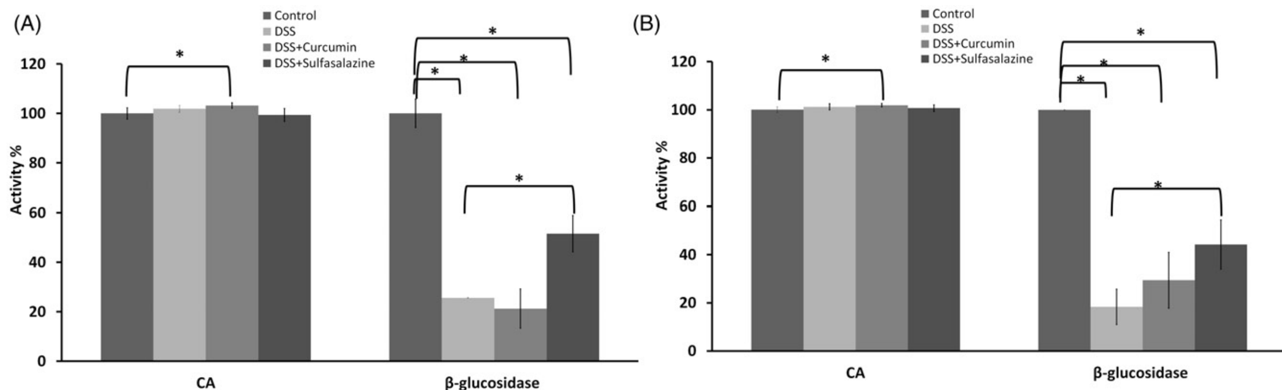

Figure 4. *In vivo* effects of DSS, DSS + curcumin and DSS + sulfasalazine on CA and cytosolic β-glucosidase activity of DSS-induced colitis mice for (A) prophylactic and (B) therapeutic application.CA enzyme activity was determined from blood. Blood samples were taken to dry tubes and centrifuged at 1500 rpm for 15 min. The packed red cells were hemolysed with cold water. Hemolysate was used for CA enzyme assay. Liver samples were used for the determination of β-glucosidase activity. Livers were powdered with liquid nitrogen, placed in 2 vol. of ice-cold extraction buffer and used for β-glucosidase assay. The data represent means ± SD ($n = 7$ mice/group). All rates were determined at least in duplicate. *$p < 0.05$.

Deguchi et al.[58] In parallel to their reports, body weight was found significantly lower in DSS-treated mice than curcumin and sulfasalazine treated mice for the therapeutic group. For the prophylactic group, weight loss of DSS-treated mice was not found to be significantly lower than the mice in the curcumin treated group (Figure 2A). As distinct from therapeutic group, the mice in the prophylactic group were administered curcumin by oral gavage and drinking water for the first seven days. Following this, they were exposed to 5% DSS in drinking water and continued to the administration of curcumin by oral gavage. Even colon length shortening was intensive in the DSS treated and curcumin treated mice compared to the control groups, the difference between the DSS and curcumin treated mice was not found to be statistically significant (Figure 2B).

Over the last few decades, there is an increasing knowledge on the role of antioxidant and anti-inflammatory properties of curcumin related to IBD[4,6]. Several pathways have been proposed to clarify the antioxidant and anti-inflammatory activities of curcumin in chronic diseases[4]. It was suggested that curcumin may block the production of pro-inflammatory biomarkers such as TNF and the pathways mediated by TNF in different cells[59–61]. The effect of curcumin on the transcriptional gene regulation to increase the anti-inflammatory cytokine IL-10 was also reported by McCann et al. in 2014[62]. Nrf2-keap1 pathway, p38- and JNK-MAPK pathways are also found to be involved in curcumin mediated decrease in inflammatory reactions[60,63]. Regarding the antioxidant properties of curcumin and the functions of inflammation related enzymes PON, CA and G6PD in the acute-phase response, the relationship between antioxidant enzymes and IBD is under particular focus of interest. As none of the studies have underlined the prophylactic effect of curcumin on PON activity, we focused on the therapeutic and prophylactic effect of curcumin on the serum and liver PON activities of colitis mice. DSS treatment exhibited statistically significant reduction in the serum and liver PON activity compared to control groups but curcumin treatment abolished the reduction in PON activity.

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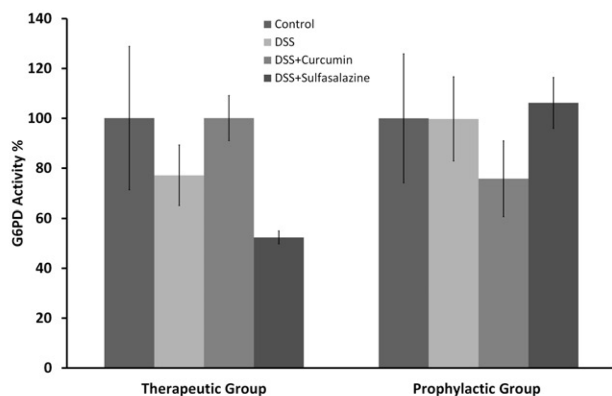

Figure 5. *In vivo* effects of DSS, DSS + curcumin and DSS + sulfasalazine on G6PD activity of DSS-induced colitis mice for prophylactic and therapeutic application. G6PD activity was determined from blood. Blood samples were taken to dry tubes and centrifuged at 1500 rpm for 15 min. The packed red cells were hemolysed with cold water. Hemolysate was used for G6PD enzyme assay. The data represent means ± SD ($n = 7$ mice/group). All rates were determined at least in duplicate. $*p < 0.05$.

Specifically prophylactic group showed significantly higher serum and liver PON activity compared to therapeutic group indicating the prophylactic effect of curcumin is noteworthy (Figure 3). Prodrugs of 5-aminosalicyclic acid (5-ASA), such as sulfasalazine, are used for the treatment of IBD for decades[64]. In our study, sulfasalazine was also used for the treatment of IBD and to compare the results with curcumin. Sulfasalazine treatment did not affect the liver PON activity compared to DSS group, but caused increase in serum PON activity compared to DSS group (Figure 3). Consistent with our results, the study indicating the effects of curcumin in gastric ulcer rats, Tuorkey and Karolin demonstrated that curcumin treated rats has statistically significant higher PON1 activity compared to rats in control group[57]. The clinical study comparing the PON activities of UC patients and healthy control group also showed that healthy individuals have higher serum PON1 activity than UC patients[65]. The importance of curcumin on PON1 transactivation was demonstrated by Schrader and colleagues using Huh7 hepatoma cell line that stably transfected with PON1 promoter. Curcumin caused dose dependent increase in PON1 transactivation, however similar stimulation was not observed in mRNA and protein levels of hepatic samples of mice that were fed with curcumin supplemented diet[66]. Curcumin supplementation on high-fat diet of hamsters also resulted increase in high-density lipoprotein cholesterol and the activity of plasma PON levels compared to control group[67].

Low intra-colonic pH and unbalanced ionic exchanges are the important alterations in UC. CAs are important enzymes regulating the colonic electrolyte transport and pH[20]. CAs generally control the bulk of carbon dioxide exchange between blood and tissues. As CAs are key enzymes for the regulation of proton and other ion movements, there are several *in vitro* and *in vivo* studies showing the inhibitory effects of different drugs on CA activity. Fonti and colleagues reported that CA activity in the colonic mucosa of mild/moderate UC patients were found lower compared to control group. Consistent with CA activity, CA-I mRNA and protein expression levels were also decreased in UC patients[20]. To our knowledge, there is not any report related to effect of curcumin on erythrocyte CA activity of UC-mice for prophylactic and therapeutic administrations. Our study shows for the first time that erythrocyte CA activity of DSS-induced colitis mouse is not significantly different from the non-treated controls for both therapeutic and prophylactic groups, but the CA activity of curcumin treated mice is significantly higher than the control groups (Figure 4).

β-Glucosidase is another remarkable inflammation related enzyme that may lead to changes in the colon. There are several studies indicating the correlation between inflammation of the colon and activity of β-glucosidase and β-glucuronidase enzymes. Analysis of β-glucosidase activity in stool specimens of children with IBD showed no significant differences from healthy control groups[28,68–70]. Prebiotic treatments are thought to be a good strategy to prolong the remission period in UC. The study focused on the prebiotic treatment in experimental colitis showed that mice that were fed with diet supplemented with 10% prebiotic product germinated barley foodstuff have higher cecal β-glucosidase activity compared to control group[28]. Liver was indicated as the major organ responsible for metabolism of curcumin but there is not any study showing the effect of curcumin on hepatic cytosolic β-glucosidase in DSS-induced colitis mice[7,71–73]. In our study, DSS treatment inhibited the hepatic cytosolic β-glucosidase activity >70% compared to control group in both the therapeutic and prophylactic groups. Inhibition of cytosolic β-glucosidase activity was slightly elevated by sulfasalazine treatment, however no significant variation was observed by curcumin compared to DSS group (Figure 4). The inhibitory effect of dextran sulfate on acid β-glucosidase was also reported by Shafit-Zagardo and Turner in human placental samples[74]. G6PD enzyme activity levels were not found significantly different among the groups used in this study (Figure 5). Consistent with our findings, hepatic G6PD activity of mice fed with curcumin supplemented high-fat diet was found to be higher than the control group but increase in G6PD was not statistically significant[67].

Conclusion

In conclusion, comparison of the prophylactic and therapeutic administration of curcumin in a mice model of UC indicated that prophylactic administration of curcumin has substantial effects on serum and liver PON activity, erythrocyte CA activity, and hepatic cytosolic β-glucosidase activity. In addition, loss of body weight loss and the shortening of the colon length in prophylactic group were also found less than in the therapeutic group. Combined with previously identified effects of curcumin in IBDs, our results on the prophylactic and therapeutic effects of curcumin will be helpful for further development of novel anti-IBD strategies.

Declaration of interest

The authors report no declarations of interest. This work was supported by a grant (2014/55) from Balikesir University Research Foundation.

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