Available online at www.sciencedirect.com

SCIENCE DIRECT®

Journal of Nutritional Biochemistry 16 (2005) 297–304

Journal of Nutritional Biochemistry

Antioxidants as novel therapy in a murine model of colitis☆

Helieh S. Oz[a,*], Theresa S. Chen[b], Craig J. McClain[b,c,d], Willem J.S. de Villiers[a]

[a]*Department of Internal Medicine, Division of Digestive Diseases and Nutrition, University of Kentucky Medical Center, Lexington KY 40536, USA*
[b]*Department of Pharmacology/Toxicology, University of Louisville, Louisville KY 40536, USA*
[c]*Department of Medicine, Division of Digestive Health and Nutrition, University of Louisville Medical School, Louisville KY 40102, USA*
[d]*VAMC, Louisville KY, USA*

Received 9 April 2004; received in revised form 1 September 2004; accepted 3 September 2004

Abstract

Reactive oxygen species (ROS) are increased in inflammatory bowel disease (IBD) and have been implicated as mediators of intestinal inflammation. We investigated the hypothesis that antioxidants with diverse properties attenuate disease progression in a murine dextran sodium sulfate (DSS)-induced colitis model. These antioxidants were (A) *S*-adenosylmethionine, a glutathione (GSH) precursor; (B) green tea polyphenols, a well-known antioxidant; and (C) 2(*R*,*S*)-*n*-propylthiazolidine-4(*R*)-carboxylic acid (PTCA), a cysteine prodrug, involved in GSH biosynthesis. BALB/c mice were divided into four groups and provided with the above mentioned antioxidants or the vehicle incorporated into chow. The animals were further divided into two subgroups and given normal drinking water (control) or water supplemented with DSS (to induce colitis), and the progression of the disease was studied. DSS-treated mice developed severe colitis as shown by bloody diarrhea, weight loss and pathological involvement ($P<.001$). However, all the antioxidants significantly improved diarrhea and colon lesions ($P<.01$), and increased body weights ($P<.05$). Hematocrits were significantly less affected in DSS-treated animals receiving antioxidants ($P<.01$). Colon lengths were significantly decreased due to mucosal inflammation in DSS-treated animals, but antioxidant therapy normalized this pathological finding ($P<.001$). The blood level of reduced GSH was decreased in DSS-treated mice ($P<.05$) and returned to normal when treated with antioxidants. Serum amyloid A (acute phase protein; $P=.0015$) and tumor necrosis factor-α (TNF-α; pro-inflammatory cytokine; $P<.01$) were significantly increased in DSS-treated animals (161 ± 40 pg/ml) and improved with antioxidant treatment ($P<.01$). Finally, actin cytoskeleton was distorted and fragmented in the mucosa of DSS-treated mice and improved with antioxidant therapy. In conclusion, three structurally dissimilar antioxidants provided protection against DSS-induced colitis in this murine model, supporting a possible role for antioxidant therapy in IBD patients.

Keywords: IBD; SAMe; Green tea polyphenols; PTCA

1. Introduction

Oxidant-mediated injury plays an important role in the pathophysiology of inflammatory bowel disease (IBD), both ulcerative colitis and Crohn's disease. Inflamed gut from IBD patients is rich in activated macrophages and neutrophils, and these cells generate excess amounts of reactive oxygen species (ROS) with subsequent increase in oxidative stress [1]. The increased generation of highly toxic ROS exceeds the limited intestinal antioxidant defense system, thereby contributing to intestinal oxidative injury in ulcerative colitis patients [2]. Excessive production of ROS has been demonstrated in circulating phagocytic and polymorphonuclear leukocyte cells in IBD patients using a chemiluminescence assay [3] and following stimulation with a bacterial chemotactic peptide, *N*-formyl-methionyl-leucyl-phenylalanine (fMLP) [4].

The tripeptide glutathione (GSH) is the most important intracellular defense against oxidative stress and is essential for both the functional and structural integrity of the gut. Glutathione may be depleted during inflammatory illness, and GSH-deficient mice show severe degradation of the

☆ This research was supported by the Martha H. Stege Endowment Fund 423574 (H. Oz), National Institutes of Health grant AT1490-01A1 (H. Oz). This manuscript was partially presented at the Nutrition Week 2004, the Annual American Society of Parental Nutrition, Las Vegas, NV, at NIH-supported Intestinal Failure Session, and was recognized with an award.

* Corresponding author. Tel.: +1 859 323 4933x81741.
E-mail address: helieh.oz@uky.edu (H.S. Oz).

0955-2863/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2004.09.007

jejunum and colonic mucosa and have body weight loss and diarrhea [5]. Administration of *N*-acetylcysteine (NAC) in the trinitrobenzene sulfonic acid (TNBS) rat model attenuated the chemically induced colitis [6], suggesting that GSH precursors may be beneficial in the acute relapse of IBD. *S*-Adenosylmethionine (SAMe), a GSH precursor, is required for the metabolism of nucleic acids, methylation reactions, polyamine production and the maintenance of the structure and function of membranes [7,8]. Green tea polyphenols (GrTP) are known antioxidants [9] that prevent the activation or transcription of NF-κB and inducible kinase (IKK) in fetal rat intestinal epithelial cells [10] and in the IL-2-deficient mouse model of autoimmunity and IBD [11]. Finally, 2(*R*,*S*)-*n*-propylthiazolidine-4(*R*)-carboxylic acid (PTCA) is a cysteine prodrug that stimulates GSH biosynthesis [12]. In the present study we investigated whether these antioxidants (SAMe, PTCA and GrTP) with diverse properties attenuate disease progression in dextran sodium sulfate (DSS)-induced colitis in mice, a well-established model of colonic inflammation. Similar to IBD, DSS treatment provokes inflammation and macrophage activation with subsequent loss of epithelial integrity and increases luminal Gram-negative flora [13].

2. Materials and methods

The antioxidant, PTCA, was synthesized and kindly provided by Dr. Herbert T. Nagasawa [12]. PTCA is an L-cysteine prodrug with masked sulfhydryl groups to stabilize it against air oxidation in the form of thiazolidine-4-carboxylic acids. *S*-Adenosylmethionine, as its 1,4-butanedisulphonate salt, was provided by Drs. R. O'Brian (Knoll Pharmaceuticals, Piscataway, NJ) and G. Stramentionoli (Knoll Farmaceutici, Milan, Italy). Green tea polyphenols were purchased from LKT laboratories (St. Paul, MN). Other chemicals were obtained from Sigma (St. Louis, MO).

2.1. Animals

BALB/C male mice 6–8 weeks of age were purchased from Sprague–Dawley (Indianapolis, IN) and were housed in micro-filter top cages at the University of Louisville, Animal Research Resources Center. They were maintained at 22 °C with a 12:12-h light/dark cycle and fed rodent chow and water ad libitum. This experimental study was approved and performed in accordance with the guidelines for Institutional Animal Care and Use Committee (IACUC), the University of Louisville Research Resource Facility, Louisville, KY, which is certified by the American Association of Accreditation of Laboratory Animal Care (AAALAC).

2.2. Experimental procedures

After an acclimatization period, mice were divided into four groups, and each group was provided with different antioxidant compounds or vehicle (sucrose) incorporated into rodent chow as follows: group A, control was provided with chow supplemented with the vehicle (sucrose). Group B received the GSH precursor, SAMe, that plays an important role in methylation reactions and polyamine production. Group C was provided with GrTP. Group D received a cysteine prodrug, PTCA, that stimulates GSH biosynthesis. Animals remained on the assigned diets until they were euthanized on day 10.

2.3. Colitis induction

Three days after the initiation of the experiment, the animals were further divided into two subgroups and given normal drinking water (normal controls) or water containing 4% DSS (ICN Biochemical, OH). Animals provided with DSS will develop a severe colitis after a period of 1 week. DSS-induced colitis is a well-accepted intestinal inflammation model and provokes colonic inflammation and macrophage activation with subsequent loss of epithelial integrity [13] The mice were randomized into subgroups (nine per group) as follows:

Normal water:

(1) Control BALB/c mice with sucrose.
(2) Control BALB/c mice treated with SAMe.
(3) Control BALB/c mice treated with GrTP.
(4) Control mice treated with PTCA.

DSS treated:

(5) DSS-treated BALB/c mice.
(6) DSS-treated BALB/c mice with SAMe.
(7) DSS-treated BALB/c mice with GrTP.
(8) DSS-treated BALB/c mice with PTCA.

2.4. Evaluation of colitis

The progression of colitis was evaluated over a period of 1 week on the DSS treatment. Parameters monitored daily were body weight, physical appearance, food consumption, presence of gross blood in stool and consistency of feces and diarrhea.

2.5. Tissue collection and histology

On day 10 after the initiation of the experiment, the animals were anesthetized with halothane inhalation. A portion of the liver, spleen, heart, small and large intestine were excised and processed for histopathological analysis. The intestines were removed and perfused with PBS, pH 7.4. A small cuff of the proximal and distal colon (within 1 cm from the rectum) was cut and fixed in 10% buffered formalin in PBS (Sigma). Liver and pancreas tissues were also subjected to the same procedure. The rest of the large and a portion of the small intestine (ileum) were dissected, perfused with PBS and flash frozen in liquid nitrogen for further analysis of the tissue antioxidants and stored at −80 °C.

The formalin-fixed sections were stained with hematoxylin and eosin (H&E) and evaluated by light microscopy for the presence of lesions. The severity of colitis was assessed

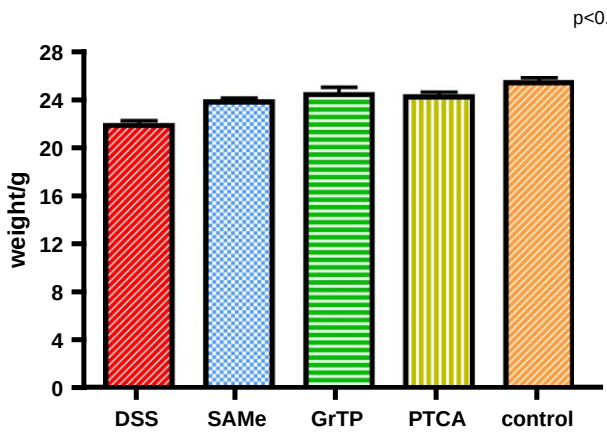

Fig. 1. Body weight (g) for the mice (mean±SEM).

by histological grading score of the colon. The scores were based on histological features with a numeric value (0–4) assigned to the specimen based on the following criteria:

Grade 0—No detectable lesions, no inflammatory cells, mucosa appeared normal.
Grade 1—Few focal inflammatory infiltrate in the mucosa.
Grade 2—Mild multifocal inflammation with moderate expansion of the mucosa; crypt epithelium appears normal.
Grade 3—Moderate multifocal inflammation with moderate expansion of the mucosa, mild crypt epithelium disruption.
Grade 4—Severe diffuse inflammation with crypt epithelium disruption and ulceration.

2.6. Fluorescent staining of the actin cytoskeleton

Formalin-fixed colon sections were stained with FITC-conjugated phalloidin (specific for F-actin staining; Sigma) at 1:30 dilution for 1 h at 37 °C and then were mounted in Aquamount. The slides were analyzed using ultrahigh-resolution laser scanning confocal microscopy (LSCM; Carl Zeiss) with a ×63 oil immersion plan-apochromat objective, NA 1.4 (Zeiss). An argon laser (wavelength=488 nm) was used to examine FITC-labeled cells, and the morphology of the cytoskeleton was examined.

2.7. Whole blood and plasma isolation

Immediately after euthanasia, blood was collected from the right ventricle of the heart into a syringe containing a minute amount of heparin and placed on ice. Plasma was separated by centrifugation at 5000×*g* for 5 min at 4 °C. Samples were kept at −80°C until further analysis.

2.8. Immunoassays

The serum concentration of serum amyloid A (SAA) was determined using ELISA (Cytoscreen M SAA, Biosource, Camarillo, CA), and tumor necrosis factor-α (TNF-α), IL-1β and IL-10 levels were measured with Quantikine M (R&D System, Minneapolis, MN).

2.9. Tissue and blood preparation for antioxidant determination

Blood samples were collected in heparinized tubes and a 20% homogenate in 5% metaphosphoric acid was prepared. After standing for 30 min on ice, the homogenate was centrifuged for 10 min (10,000 *g*), and the acid-soluble fraction was collected for measurement of sulfhydryl and disulfide compounds.

Tissue homogenates (10%, w/v) were prepared in 5% (w/v) metaphosphoric acid, using all-glass Tenbroeck homogenizers, and kept on ice. After standing for 20–40 min, the homogenates were centrifuged for 1 min (10,000×*g*), and the acid soluble fractions were collected for measurement of free thiol disulfides.

2.10. Analysis of GSH and other thiols (SH) and disulfides (SS)

Reduced GSH (rGSH), GSH disulfide (GSSG), cysteine and cystine were simultaneously quantified by high-performance liquid chromatography with dual electrochemical detection (HPLC-DEC) according to the method of Richie and Lang [14] with slight modification [15]. In brief, 20-μl samples were injected onto a 250×4.6-mm, 5-μm, C-18 column (Val-U-Pak HP, fully end-capped ODS; Chrom Tech, Apple Valley, MN). Samples (20 μl) were injected onto the column and eluted isocratically with a mobile phase consisting of 0.1 M monochloroacetic acid, 2 mM heptane

Table 1
Tissue and blood values from antioxidant groups treated or untreated with DSS

Group	DSS	SAMe	GrTP	PTCA	H_2O	*P* value
Weight (g)	22.1±0.4[a,b]	24.1±0.3[a]	24.7±0.6[a]	24.5±0.4[a]	25.7±.4[b]	<0.001[b], <0.05[a]
Hematocrit	47±0.5	50±0.6	48±0.6	50±0.3	49± 0.4	*P*<0.01
Colon length (mm)	66±2.8[a,b]	80±4[b]	84±4.2[b]	87±2.6[a]	109±2.8[a]	<0.001[a], <0.05[b]
Blood rGSH[c]	881±75	1119±67	1124±54	1147.8±52	1105.9±23	0.0279
Blood GSSG[d]	63±15	48.2±10	38.7±1.9	34±1.9	49±5	0.0122
Colon rGSH[c]	1300±109[a,b]	1314±110	1267±143	2086±83[a]	1598±35[b]	>.05[b], <0.001[a]
Colon GSSG[d]	291±21[a,b]	274±15	345±23	172±7[a]	182±27[b]	<.01[b], <.001[a]

Data are presented as mean±SEM. Statistical significance is set at *P*<.05.
[a] Statistical comparison for *P* values.
[b] Statistical comparison for *P* values.
[c] Reduced glutathione.
[d] Oxidized GSH.

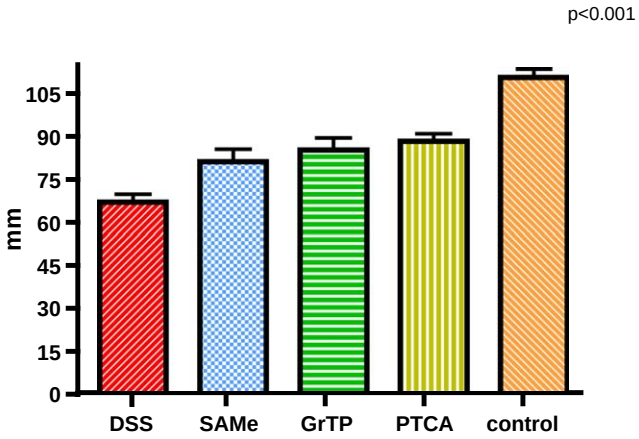

Fig. 2. Colon length (mm) for the mice (mean±SEM).

sulfonic acid and 2% acetonitrile at pH 2.8 and delivered at a flow rate of 1 ml/min. The compounds were detected in the eluant with a Bioanalytical Systems model LC4B dual electrochemical detector using two Au–Hg electrodes in series with potentials of −1.2 and 0.15 V for the upstream and downstream electrodes, respectively. Current (nA) was measured at the downstream electrode. Analytes were quantified from peak area measurements using authentic external standards.

2.11. Statistical analysis

All results are expressed as mean±SEM unless otherwise stated. Data were evaluated using analysis of variance followed by appropriate post hoc test using GraphPad Instat version 3 for Windows (GraphPad software, San Diego, CA). Statistical significance was set at $P<.05$.

3. Results

There was no significant difference noted between the body weights of control animals (no DSS) when treated with antioxidant alone. DSS-treated mice developed severe colitis and lost more body weight [DSS 22.1±0.4 g vs. control 25.7±0.4 g ($P<.001$)] compared to DSS+antioxidant-treated animals (Fig. 1). There were no differences in body weight between different antioxidant groups and controls ($P=.225$)]. The hematocrits were decreased in DSS-treated animals [DSS 47±0.5% vs. control 49±0.4% ($P<.01$)] and improved following antioxidant therapy (Table 1).

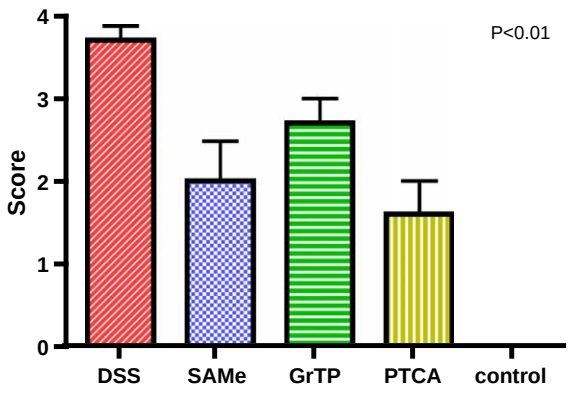

Fig. 3. Colon pathology score (0–4) (mean±SEM).

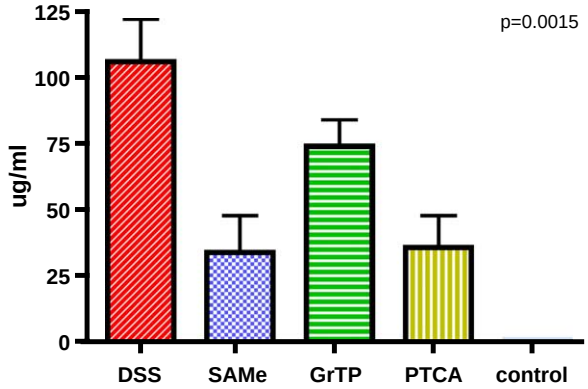

Fig. 4. Average serum amyloid A (μg/ml) detected by ELISA (mean±SEM).

Colon length was significantly decreased in DSS-treated animals due to mucosal inflammation and edema [DSS 66±2.8 mm vs. control 109±2.8 mm ($P=<.001$)], and antioxidant therapy improved this abnormality (Fig. 2). There was no significant differences between different antioxidant-treated groups ($P=.41$). Colon lesions (score, 0–4) were significantly improved in treated animals compared to DSS mice [DSS 3.7±0.2; SAMe 2±0.4; GrTP2.7±0.3; PTCA 1.6±0.4 ($P<.01$)] (Fig. 3). Serum amyloid A levels were significantly increased in DSS mice (106±16 μg/ml) and suppressed in antioxidant-treated animals ($P=.0015$) (Fig. 4). Serum TNF-α was significantly elevated in colitis animals (DSS 161±40 pg/ml), and this pro-inflammatory marker was drastically suppressed with antioxidant treatment ($P<.01$) (Fig. 5). There was no significant difference detected in the serum levels of IL-1β and IL-10 in the various treatment groups. Colonic rGSH was decreased in normal mice (no DSS) fed GrTP (16%) and increased in PTCA (9%)- and SAMe (16%)-treated mice compared to normal animals (control 1598±34 nmol/g; SAMe 1855±71 nmol/g; GrTP 1336±64 nmol/g;

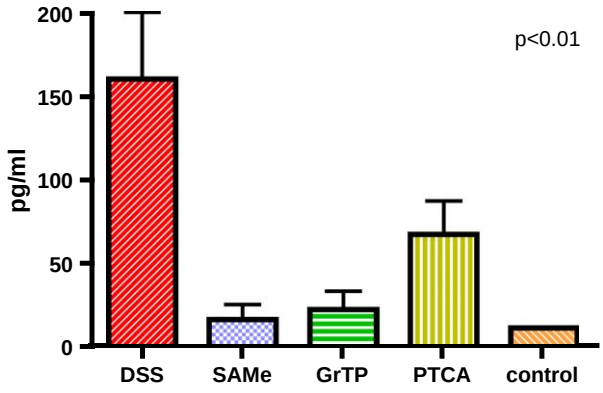

Fig. 5. Serum concentration of cytokine TNF-α (pg/ml) detected with ELISA (mean±SEM).

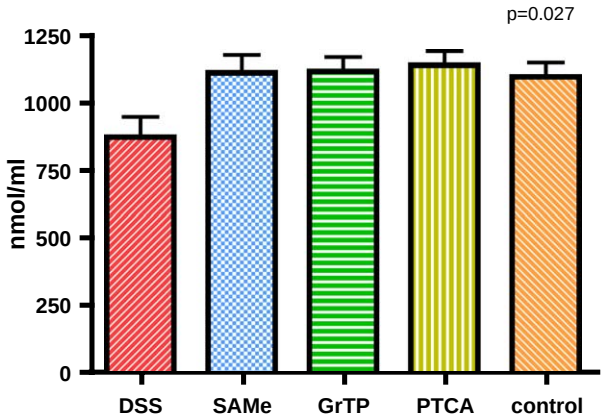

Fig. 6. Average blood level of rGSH measured by HPLC (mean±SEM).

PTCA1737±77 nmol/g). However, when animals were consequently treated with both DDS and antioxidants there was no significant difference in colonic rGSH between DSS and antioxidant-treated mice [DSS 1300±109 nmol/g vs. control 1598±35 nmol/g (P=.001); PTCA vs. DSS (P<.05)] (Table 1). The blood level of rGSH was decreased in DSS-treated mice [DSS 881±75 nmol/ml vs. control 1105.9±23 nmol/ml (P=.0279)] but was restored to normal levels in antioxidant-treated mice (Fig. 6). The blood ratio of reduced-to-oxidized GSH (% rGSH/GSSG) was significantly decreased in DSS-treated mice compared to controls (DSS 1.59, control 2.09), and this ratio improved significantly in antioxidant-treated animals (SAMe 2.08; GTP 2.55; PTCA 2.94). Finally, fluorescent staining for the actin cytoskeleton showed distortion and fragmentation in the intestinal epithelium and brush border of the DSS-treated mice. However, colons from animals treated with antioxidants showed more normal, round, smooth and continuous cytoskletal architectures (Fig. 7).

4. Discussion

The purpose of the present investigation was to determine the efficacy of three different antioxidants with dissimilar properties against colitis in a rodent model of DSS-induced colitis.

Reactive oxygen species have been implicated to contribute to tissue destruction in IBD. These ROS include hydroxyl radicals, superoxide anion, hydrogen peroxide and nitric oxide. Reactive oxygen species are extremely unstable species due to the high reactivity that may result in lipid peroxidation and the oxidation of DNA and proteins [16]. Antioxidant status has been described to be compromised in the intestinal mucosa of patients with ulcerative colitis [17,18]. In vitro studies using enterocytes have also demonstrated the damaging effects of exposure to oxidants [19]. Similar studies reveal that the colons of IBD patients produce more oxygen free radicals compared with those of control subjects [3,4]. In addition, using chemiluminescence assay, significantly elevated levels of reactive oxygen metabolites were found in actively inflamed mucosa of IBD patients [20].

Glutathione is the most abundant cellular antioxidant synthesized by animal cells. It plays an essential role in cell biology and modulates cell response to redox changes

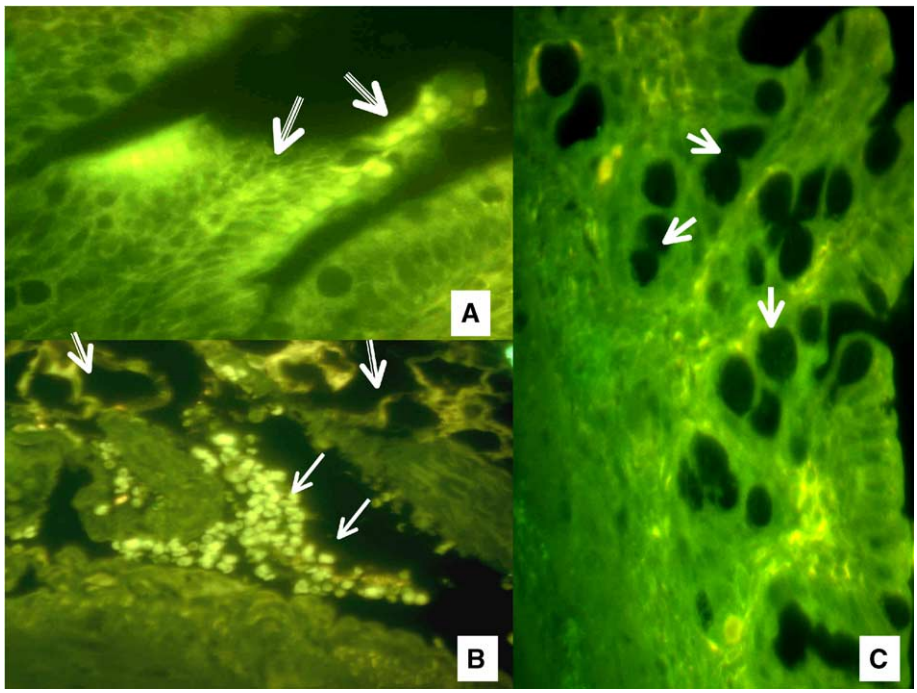

Fig. 7. Fluorescent staining of the actin cytoskeleton from DSS (A–B) showing altered cytoskeletal structure of intestinal epithelium, destruction of brush border cytoskeleton and villi; distortion and fragmentation of cytoskletal structure and actin disruption (⇧) followed by infiltration of polymorphonuclear cells (↑) in DSS mice. (C) PTCA-treated colon demonstrates (🡻) more normal, round, smooth and continuous cytoskeletal architecture.

associated with the ROS. Glutathione may be depleted during inflammatory illness, and GSH deficiency predisposes animals to organ failure and death after an otherwise nonlethal period of hypotension [21]. Because GSH deficiency is associated with severe injury such as inflammation and sepsis, treatment strategies that maintain GSH stores may decrease the incidence of organ failure. Depletion of GSH induced enlarged lymphoid aggregates in the intestine by recruitment of lymphocytes from the peripheral circulation [22]. This depletion in tissue levels of GSH has been implicated as a component of the inflammation that develops in IBD patients. On the other hand, supplementation with GSH monoethylester has been reported to prevent these lymphoid aggregates [22]. Accordingly, administration of NAC in the TNBS rat model attenuated the chemically induced acute colitis through increased mucosal GSH levels, suggesting that GSH precursors may be of relevance in the acute relapse of IBD [6].

In addition, toxic bacterial metabolite sulfide is reported to be involved in the pathogenesis of ulcerative colitis. Colitis patients taking 5-aminosalicylic acid (5-ASA)-containing drugs have lower fecal sulfide levels than those not taking these drugs. Inhibition of sulfide production by 5-ASA-containing drugs has been implemented to their therapeutic effect [23].

DSS-treated rats showed an increase in plasma-oxidized proteins indicating that DSS induced oxidative stress at the systemic level that persisted until the end of the treatment [24]. Oxidative stress is linked with the stimulation of the immune system [25]. Rebamipide, a drug that inhibits the production of free radicals, was shown to act as an anti-inflammatory agent in chronic DSS-induced colitis [26]. In the present investigation, DSS-treated animals developed severe colitis and lost a significant amount of body weight. These animals also had decreased hematocrits, decreased rGSH concentrations in the blood and colon, and elevated inflammatory markers such as serum amyloid A and TNF-α when compared to normal controls. In the current study there was no significant difference between liver rGSH and GSSG in DSS-induced colitis compared to normal (H_2O) controls [rGSH (nmol/g): DSS 5245±131; control 5342±175 (P=.078)]. However, oxidized disulfide (GSSG) in the colon was significantly increased in DSS-induced mice when compared to normal animals (P<.01). The control of oxidative stress involves multiple antioxidant mechanisms. The cysteine precursor PTCA has been shown to protect against acetaminophen (APAP)-induced hepatic GSH, cysteine depletion [27,28] and hepatic necrosis [29]. The results demonstrated a high degree of tissue selectivity in the APAP-induced depletion of GSH and cysteine concentrations, and in the effectiveness of PTCA in maintaining and even elevating sulfhydryl levels in extrahepatic tissues of APAP-treated mice, while the protective effect of PTCA was related to prevention of hepatic sulfhydryl depletion. Other study also showed that PTCA significantly increases GSH biosynthesis in cultured rat lens exposed to ultraviolet and radiation as compared to untreated controls [30]. In this study, PTCA-treated mice had significantly improved hematocrit, colon length and colon lesions with higher levels of blood rGSH compared to DSS-treated animals. In addition, PTCA animals also showed higher levels of rGSH and lower GSSG concentrations in their liver and colon when compared to untreated controls.

Ulcerative colitis is associated with a selective reduction of *n*-butyrate oxidation by the colonic epithelial cells, although the mechanism for this is unclear. Bacterial production of anionic sulfide is reported to be increased in the colon of ulcerative colitis patients, and these sulfides can cause metabolic damage to colonocytes [31]. Sulfide toxicity in isolated colonocytes can be reversed by methyl donors. *S*-Adenosylmethionine is an obligatory intermediate in the conversion of methionine to cysteine in the transsulfuration pathway. The conversion of methionine to SAMe involves the transfer of the adenosyl moiety of ATP to the sulfur atom of methionine [32]. *S*-Adenosylmethionine serves as an antioxidant not only because of its role as a precursor for GSH biosynthesis, but also because of its capacity to interact directly with ROS. Evidence to support this role comes from studies on the capacity of SAMe to diminish ischemia/reperfusion injuries in clinical trials during liver transplantation [33]. Ulcerative colitis is also associated with a selective reduction of *n*-butyrate oxidation by the colonic epithelial cells [31]. The reasons for this action are not completely understood. Incubation of colonic cell suspension with sulfide induced a significant inhibition of $^{14}CO_2$ production as compared with controls. With the addition of SAMe 1,4 butane disulfonate, cell suspension reversed this effect in proximal but not in distal cell incubations, suggesting a greater susceptibility of the distal colon to the sulfide effect [34]. The role of mucosal detoxification of sulfide by thiolmethyltransferase (TMT)-mediated methylation was suggested in protecting the healthy colonic mucosa from the adverse effects of luminal sulfide. In the human colon, methylation appears to protect colonic epithelial cells against sulfide-induced inhibition of *n*-butyrate oxidation. This action cannot be directly correlated with mucosal TMT activity [35].

Luminal anionic sulfide may contribute to epithelial damage in ulcerative colitis. Erythrocyte TMT activity was elevated after proctocolectomy for Crohn's disease and ulcerative colitis. In patients with active stage of ulcerative colitis, the mucosal spermidine concentration was increased due to exogenous uptake in comparison with patients in remission or in healthy controls. The activities of ornithine decarboxylase and *S*-adenosylmethionine decarboxylase, rate-limiting enzymes of polyamine biosynthesis, were lower in the patients with ulcerative colitis [36]. These findings in ulcerative colitis and our results in the current DSS-induced colitis study suggest that sulfide detoxification may be important in the pathogenesis and treatment of colitis and that SAMe may have a therapeutic value.

Previously, we reported that GrTP attenuates inflammation in the IL-2-deficient mice model of autoimmunity and IBD [11]. Following ingestion, GrTP is widely distributed throughout the body with one of the highest tissue concentrations found in the intestine [37]. The anti-inflammatory effects of green tea have been attributed to polyphenol fraction, which is rich in antioxidants [9]. The effects of GrTP may not be limited to the scavenging of toxic oxidants. We have shown that GrTP and, specifically, the polyphenols EGCG block the activation of the transcription factor, nuclear factor-κB (NF-κB), in intestinal epithelial (IEC-6) cells [10]. NF-κB plays a critical role in the pathogenesis of chronic inflammatory diseases and in IBD patients [38]. Inhibitors of NF-κB have been shown to decrease inflammation in the animal model of IBD confirming its pivotal role [39]. The ability of GrTP to inhibit NF-κB activation may in part be responsible for its anti-inflammatory effects. In the current study, GrTP significantly improved the colon length and blood rGSH, and lowered TNF-α and SAA levels when compared to DSS-treated animals that had not received any antioxidant therapy. However, to our surprise, GrTP treatment (alone) significantly lowered rGSH and GSSG levels in the liver and the colon suggesting that antioxidant GrTP effects may also be related to other mechanisms in addition to blocking NF-κB activation. Based on our findings, that antioxidants reduce disease activity in the murine model of DSS-induced colitis, we postulate that these antioxidants may also prove to be a useful dietary supplement in the treatment of IBD in humans.

5. Conclusions

Three structurally dissimilar antioxidants provided protection in this experimental rodent model of colitis, supporting a possible role for antioxidant therapy in IBD patients. In addition, some of these compounds may have other properties with additive or synergistic effects that need to be explored.

Acknowledgment

PTCA was synthesized and kindly provided by Dr. Herbert T. Nagasawa from the Department of Medicinal Chemistry, University of Minnesota. Marcia C. Liu provided technical assistance.

References

[1] Grisham MB, Granger DN. Neutrophil-mediated mucosal injury. Role of reactive oxygen metabolites. Dig Dis Sci 1988;33:6S–15S.
[2] Babbs CF. Oxygen radicals in ulcerative colitis. Free Radic Biol Med 1992;13:169–81.
[3] Suematsu M, Suzuki M, Kitahora T, Miura S, Suzuki K, Hibi T, et al. Increased respiratory burst of leukocytes in inflammatory bowel diseases—the analysis of free radical generation by using chemiluminescence probe. J Clin Lab Immunol 1987;24:125–8.
[4] Shiratora Y, Aoki S, Takada H, Kiriyama H, Ohto K, Hai K, et al. Oxygen-derived free radical generating capacity of polymorphonuclear cells in patients with ulcerative colitis. Digestion 1989;44:163–71.
[5] Mrtensson J, Jain A, Meister A. Glutathione is required for intestinal function. Proc Natl Acad Sci U S A 1990;87:1715–9.
[6] Ardite E, Sans M, Panes J, Romero FJ, Pique JM, Fernandez-Checa JC. Replenishment of glutathione levels improves mucosal function in experimental acute colitis. Lab Invest 2000;80:735–44.
[7] Giulidori P, Galli-Kienle M, Catto E, Stramentinoli E. Transmethylation, transsulfuration, and aminopropylation reactions of *S*-adenosyl-L-methionine in vivo. J Biol Chem 1984;259:4205–11.
[8] Lu SC. *S*-Adenosylmethionine. Int J Biochem Cell Biol 2000;32:391–5.
[9] Khan SG, Katiyar SK, Agarwal R, Mukhtar H. Enhancement of antioxidant and phase II enzymes by oral feeding of green tea polyphenols in drinking water to SKH-1 hairless mice: possible role in cancer chemoprevention. Cancer Res 1992;52:4050–2.
[10] Yang F, Oz HS, Barve S, de Villiers WJ, McClain CJ, Varilek GW. The green tea polyphenol (−)-epigallocatechin-3-gallate blocks nuclear factor-kappa B activation by inhibiting I kappa B kinase activity in the intestinal epithelial cell line IEC-6. Mol Pharmacol 2001;60:528–33.
[11] Varilek GW, Yang F, Lee EY, deVilliers WJ, Zhong J, Oz HS, et al. Green tea polyphenol extract attenuates inflammation in interleukin-2-deficient mice, a model of autoimmunity. J Nutr 2001;131:2034–9.
[12] Nagasawa HT, Goon DJ, Muldoon WP, Zera RT. 2-Substituted thiazolidine-4(*R*)-carboxylic acids as prodrugs of L-cysteine, protection of mice against acetaminophen hepatotoxicity. J Med Chem 1984;27:591–6.
[13] Kim HS, Berstad A. Experimental colitis in animal model. Scand J Gastroenterol 1992;27:529–37.
[14] Richie Jr JP, Lang CA. The determination of glutathione, cyst(e)ine, and other thiols and disulfides in biological samples using high performance liquid chromatography with dual electrochemical detection. Ann Biochem 1987;163:9.
[15] Chen TS, Richie Jr JP, Lang CA. Life span profiles of glutathione and acetaminophen detoxification. Drug Metab Dispos 1990;18:882–7.
[16] Beckman KB, Ames BN. Oxidative decay of DNA. J Biol Chem 1997;272:19633–6.
[17] Parks DA, Bulkley GB, Granger DN. Role of oxygen-derived free radicals in digestive tract diseases. Surgery 1983;94:415–22.
[18] Tsuchiya M. Free radicals in digestive diseases. Proceedings of the 1st International Symposium on Free Radicals in Digestive Diseases. New York: Excerpta Medica; 1988. p. 244.
[19] Baker SS, Campbell CL. Enterocyte injury by O_2-dependent processes. Gastroenterology 1989;96:A23 [abstract].
[20] Simmonds NJ, Allen RE, Stevens TR, Van Someren RN, Blake DR, Rampton DS. Chemiluminescence assay of mucosal reactive oxygen metabolites in inflammatory bowel disease. Gastroenterology 1992;103:186–96.
[21] Robinson MK, Rounds JD, Hong RW, Jacobs DO, Wilmore DW. Glutathione deficiency increases organ dysfunction after hemorrhagic shock. Surgery 1992;112:140–7, 148–9.
[22] Koch TR, Yuan LX, Fink JG, Petro A, Opara EC. Induction of enlarged intestinal lymphoid aggregates during acute glutathione depletion in a murine model. Dig Dis Sci 2000;45:2115–21.
[23] Edmond LM, Hopkins MJ, Magee EA, Cummings JH. The effect of 5-aminosalicylic acid-containing drugs on sulfide production by sulfate-reducing and amino acid-fermenting bacteria. Inflamm Bowel Dis 2003;9:10–7.
[24] Mercier S, Breuillé D, Mosoni L, Obled C, Mirand PP. Chronic inflammation alters protein metabolism in several organs of adult rats. J Nutr 2002;132:1921–8.
[25] Dieleman LA, Palmen MJ, Akol H, Bloemena E, Pena AS, Meuwissen SGM, et al. Chronic experimental colitis induced by dextran sulfate sodium (DSS) is characterized by Th1 and Th2 cytokines. Clin Exp Immunol 1998;114:385–91.

[26] Iwai A, Iwashita E. Changes in colonic inflammation induced by dextran sulfate sodium (DSS) during short- and long-term administration of rebamipide. Dig Dis Sci 1998;43:143S–7S.
[27] Srinivasan C, Williams WM, Nagasawa HT, Chen TS. Effects of 2(*RS*)-*n*-propylthiazolidine-4(*R*)-carboxylic acid on extrahepatic sulfhydryl levels in mice treated with acetaminophen. Biochem Pharmacol 2001;61:925–31.
[28] Srinivasan C, Williams WM, Ray MB, Chen TS. Prevention of acetaminophen-induced liver toxicity by 2(*R*,*S*)-*n*-propylthiazolidine-4(*R*)-carboxylic acid in mice. Biochem Pharmacol 2001;61:245–52.
[29] Oz HS, McClain CJ, Nagasawa H, Mukunda R, Chen TS. Diverse antioxidants protect against acetaminophen hepatotoxicity. J Biochem Mol Tox 2005;18:361–8.
[30] Holleschau AM, Rathbun WB, Nagasawa HT. An HPLC radiotracer method for assessing the ability of L-cysteine prodrugs to maintain glutathione levels in the cultured rat lens. Curr Eye Res 1996;15:510.
[31] Roediger WE, Babidge W, Millard S. Methionine derivatives diminish sulphide damage to colonocytes — implications for ulcerative colitis. Gut 1996;39:77–81.
[32] Mato JM, Alvarez L, Ortiz P, Pajares MA. *S*-Adenosylmethionine synthesis: molecular mechanisms and clinical implications. Pharmacol Ther 1997;73:265–80.
[33] Dunne JB, Piratvisuth T, Williams R, Tredger JM. Treatment of experimental ischemia/reperfusion injury with *S*-adenosylmethionine: evidence that donor pretreatment complements other regimens. Transplant 1997;63:500–6.
[34] Moore JW, Babidge WJ, Millard SH, Roediger WE. Thiolmethyltransferase activity in the human colonic mucosa: implications for ulcerative colitis. J Gastroenterol Hepatol 1997;12:678–84.
[35] Roediger WE, Babidge WJ. Thiol methyltransferase activity in inflammatory bowel disease. Gut 2000;47:206–10.
[36] Obayashi M, Matsui-Yuasa I, Matsumoto T, Kitano A, Kobayashi K, Otani S. Polyamine metabolism in colonic mucosa from patients with ulcerative colitis. Am J Gastroenterol 1992;87:736–40.
[37] Suganuma M, Okabe S, Oniyama M, Tada Y, Ito H, Fujiki H. Wide distribution of [^{3}H](−)-epigallocatechin gallate, a cancer preventive tea polyphenol, in mouse tissue. Carcinogenesis 1998;10:1771–6.
[38] Schreiber S, Nikolaus S, Hampe J. Activation of nuclear factor kappa B in inflammatory bowel disease. Gut 1998;42:477–84.
[39] Neurath MF, Pettersson S, Meyer-zum-Buschenfelde KH, Strober W. Local administration of antisense phosphorothioate oligonucleotides to the p65 subunit of NF-κB abrogates established experimental colitis in mice. Nat Med 1996;2:998–1004.