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Suppression of dextran sodium sulfate-induced colitis in mice by zerumbone, a subtropical ginger sesquiterpene, and nimesulide: separately and in combination

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Abstract

Ulcerative colitis (UC) and Crohn's disease are inflammatory disorders of unknown cause and difficult to treat, though some synthetic chemicals, including ligands for peroxisome proliferator-activated receptors (PPARs), are anticipated to be useful drugs. In contrast, few food phytochemicals have been reported to suppress colitis in animal models. The present study was undertaken to explore the suppressive efficacy of zerumbone (ZER), a sesquiterpenoid present in the rhizome of *Zingiber zerumbet* Smith that is used as a condiment in Southeast Asian countries and known to be a potent suppressant of cyclooxygenase (COX)-2 and inducible nitric oxide synthase expression in cell culture systems. Acute colitis was induced by exposing female ICR mice to 5% DSS in drinking water for 1 week. One week prior to DSS administration, the experimental mice were fed ZER alone, nimesulide (NIM, a selective COX-2 inhibitor) alone, or both in combination (1000 ppm each) for a total of 2 weeks. Inflammatory biomarkers, i.e. interleukin (IL)-1α and IL-1β, tumor necrosis factor (TNF)-α, and prostaglandin (PG)E_2 and $PGF_{2\alpha}$ in colonic mucosa were quantified by an enzyme-linked immunosorbent assay in conjunction with histological alterations. Oral feeding of ZER significantly lowered the levels of IL-1β [inhibitory rate (IR) = 34%], TNF-α (IR = 29%), and PGE_2 (IR = 73%) and suppressed DSS-induced colitis, whereas NIM suppressed the histological changes induced by DSS without affecting inflammatory biomarkers. However, their treatment in combination was most effective for suppressing these biomarkers. Our results suggest that ZER is a novel food factor for mitigating experimental UC and that use of a combination of agents, with different modes of actions, may be an effective anti-inflammatory strategy.

Keywords: Interleukin-1; Cyclooxygenase-2; Prostaglandin; Tumor necrosis factor-α; Inflammatory bowel disease

1. Introduction

Ulcerative colitis (UC) is an inflammatory bowel disease that approximately 60,000 patients in Japan and 500,000 in the US suffer from, though its pathogenesis is unknown. Disruption of immune systems in the intestinal tract is suggested to be involved in the etiology of UC, thus immune suppressive agents and antibiotics are currently used as tentative drugs. However, prolonged and chronic UC may progress to colorectal cancer.

Along with murine colitis models induced with 1-hydroxyanthraquinone and methylazoxymethanol acetate [1], dextran sodium sulfate (DSS)-induced models share some pathological symptoms such as diarrhea, bloody feces, body weight reduction, mucosal ulceration, and shortening of the large intestine, as well as others [2]. The DSS-induced model is easy to work with and requires only a short duration to obtain the results, thus it has been utilized to screen synthetic compounds for the regulation of colitis including UC.

Reactive oxygen and nitrogen species have been reported to be involved in the development of UC in tests

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Abbreviations: UC, uclerative colitis; PPAR, peroxisome proliferator-activated receptors; ZER, zerumbone; NIM, nimesulide; COM, combination; COX, cyclooxygenase; iNOS, inducible nitric oxide synthase; IL, interleukin; PG, prostaglandin; TNF, tumor necrosis factor.

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that modified the environmental components, including proteins and DNA bases [3,4]. Inflammatory cytokines such as interferon-γ and TNF-α have been implicated in the expression of inducible nitric oxide synthase (iNOS) [5], which generates excess amounts of nitric oxide (NO) from inflammatory leukocytes in colitis tissues [6]. Further, depletion of the activity of a phase II xenobiotic metabolizing enzyme, glutathione-*S*-transferase, was found in DSS-induced colitis [7], while increased expression levels of interleukin (IL)-1 [8] and COX-2 and resultant PGE_2 production [9–12] have been reported by several researchers and may be involved in the etiology of UC by unknown mechanism(s).

To control UC, one of the effective and promising group of compounds is the ligands for peroxisome proliferator-activated receptors (PPAR)-α and -γ, including troglitazone and bezafibrate [13]. Another group is selective COX-2 inhibitors such as nimesulide (NIM) [14,15], though contradicting data has also been presented [16]. Nevertheless, to our knowledge, there is no report of food phytochemicals suppressing DSS-induced UC in mice.

Zerumbone is a sesquiterpenoid with very large amounts detected in rhizomes, which are used locally as anti-inflammatory medicines [4], and is also present in some edible parts, including young stems and inflorescence, which are used in traditional cooking [5]. Our previous study demonstrated that ZER suppresses free radical generation, iNOS expression, and TNF-α release, and also induces apoptosis in a variety of human colonic adenocarcinoma cell lines [17]. In particular, it should be noted that ZER attenuated the expression of COX-2 in stimulated RAW 264.7 murine macrophages, but did not affect COX-2 catalytic activity [17].

The above scientific background led us to investigate the suppressive activity of ZER on DSS-induced colitis in mice and to compare its efficacy with NIM. In addition, the synergistic effect of both compounds was also addressed because they have distinct modes of action mechanisms, i.e. COX-2 expression suppression (ZER) and COX-2 catalytic activity blockade (NIM).

2. Materials and methods

2.1. Chemicals

ZER (purity > 95%) was isolated and purified from a chloroform extract of the rhizomes of *Z. zerumbet* Smith as previously reported [18]. NIM and DSS (molecular weight: 36,000–50,000) were purchased from Sigma-Aldrich and ICN Biomedicals, respectively. ELISA kits for quantifying IL-1αβ and TNF-α were obtained from Endogen Inc., and PGE_2 and $PGF_{2\alpha}$ came from Cayman. Other chemicals were purchased from Wako Pure Chemical Industries unless specified otherwise.

2.2. Animals

Female ICR mice at 7 weeks of age were obtained from Japan SLC, housed five per cage, and given fresh tap water *ad libitum* and commercial rodent pellets (MF: Oriental Yeast Co.) that were freshly changed twice a week. The mice were treated in accordance with the Guidelines for Animal Experimentation of Kyoto University. Animals were maintained in a room controlled at $24 \pm 2^\circ$ with a relative humidity of $60 \pm 5\%$ and a 12 hr light/dark cycle (06:00–18:00 hr).

2.3. DSS-induced colitis in mice

Colitis was induced by the method previously reported by Kitajima *et al*. [19] with some modifications. After a 1 week quarantine as described above, the mice were divided into five groups composed of 15 mice each. In the control group (Group 1), mice were given fresh tap water *ad libitum* and MF pellets, freshly changed twice a week, for 2 weeks. In the DSS group (Group 2), 5% DSS in tap water was given 1 week after the beginning of experiment to induce colitis, and the mice were fed with MF pellets. In other three groups, 0.1% ZER (Group 3), 0.1% NIM (Group 4), or 0.1% ZER plus 0.1% NIM (Group 5) was added to the MF pellets for 2 weeks. One week after beginning the experiment, 5% DSS drinking water was given to all mice as well as the DSS group, except for the controls. The body weight of each mouse was recorded 1, 7, and 14 days after the start of the experiment, and the intake of food and water was measured daily.

2.4. Tissue sample preparations

After the end of the experiment (week 2), all mice were killed by cervical dislocation and the large intestines without the cecum were removed. After washing in ice-cold phosphate-buffered saline (PBS), they were placed on filter papers to measure their length, after which they were opened to remove their contents. Colonic mucosa samples, scraped off by razors, were put into ice-cold 0.5 mL of PBS and homogenized on ice for 15 s. The homogenates thus obtained were centrifuged at 1900 *g* at 4° for 15 min and stored at -80° or lower until use.

2.5. ELISA

Proinflammatory mediators (IL-1α, IL-1β, TNF-α, PGE_2, and $PGF_{2\alpha}$) in the supernatants, obtained as described above, were quantified using commercial experimental kits according to the protocol of the manufacturer. The dilution factors for each biomarker were as follows: IL-1α (×1/2), IL-1β (×1/4), TNF-α (×1/2), PGE_2 (×1/200) and $PGF_{2\alpha}$ (×1/400), with dilution done with PBS.

2.6. Protein determination

Protein concentrations in each supernatant were determined using a DC Protein Assay kit (Bio-Rad Laboratories) according to the protocol of the manufacturer (dilution factor: 20), with γ-globulin employed as the standard.

2.7. Histological examination

Each excised large intestine was fixed in Mildform® (Wako Pure Chemical Industries), cut longitudinally and then embedded in paraffin. Transverse sections (3 μm) were cut and stained with hematoxylin and eosin. Sequential high-power fields of the entire colon were evaluated histopathologically. Erosion and ulcer were counted microscopically on hematoxylin and eison-stained sections from each mouse large intestine. After photographed all areas of large intestine on the sections, histological scoring was done in a blind fashion by a pathologist (T.T.) based on injury to the colonic mucosa, with particular attention paid to alterations of the colonic crypts and the presence of edema and inflammation in the colon as described previously [20]. In brief, edema scores were assessed as: Grade 0, absence of edema in the colon; Grade 1, mild edema in the mucosa; Grade 2, edema in the mucosa and submucosa; and Grade 3, edema in the entire wall of the colon. Inflammation was scored as: Grade 0, a few inflammatory cells; Grade 1, mild inflammation of the lamina propria and submucosa; Grade 2, severe inflammation of both the lamina propria and submucosa; and Grade 3, severe inflammation in the entire wall of the colon. In areas adjacent to erosions or ulcerations, cryptal regenerative changes were observed. In general, the nuclei and nucleoli were larger in regenerative cryptal cells than those in normal cryptal cells and their cytoplasm was basophilic. Such regenerative changes in the cryptal cells surrounding erosion or an ulcer were scored as: Grade 0, no regenerative changes; Grade 1, slight regenerative changes; Grade 2, moderate regenerative changes; Grade 3, severe regenerative changes; and Grade 4, severe regenerative changes with fibrosis in the mucosa.

2.8. Statistical analysis

Data are shown as mean ± SD values. The statistical significance of differences between groups in each assay was assessed by a Student's *t*-test (two-sided) that assumed unequal variance.

3. Results

3.1. General observations

No mice each group died throughout the experiments. The intake of drinking water in the four groups given DSS

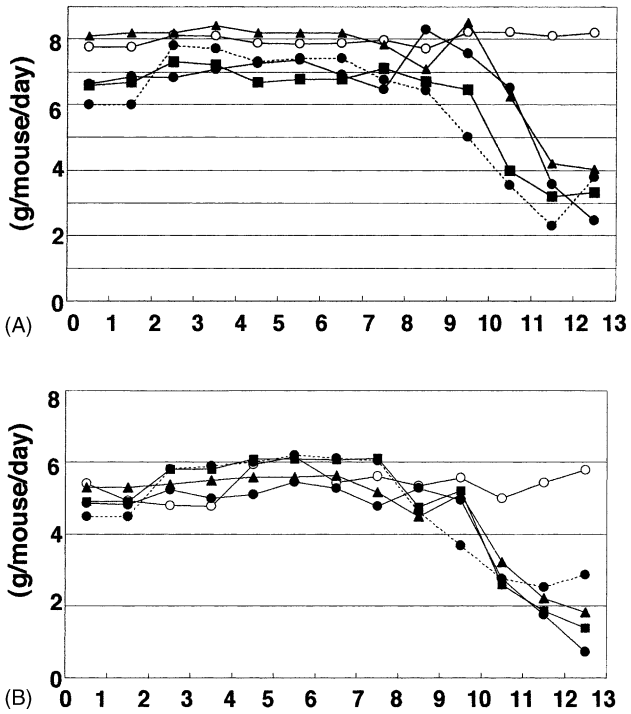

Fig. 1. Intake of drinking water (panel A) and diet (panel B) in each group. Group 1 (○–○); Group 2 (●–●); Group 3 (▲–▲); Group 4 (■–■); Group 5 (●- - -●).

(Groups 2–5) began to decrease 3–4 days after DSS administration (Fig. 1A), and there were significant differences between Group 1 (the control) and the other four groups. However, no statistical differences in the intake of drinking water were observed among the four experimental groups. Food intake among the four groups who received DSS also declined 3–4 days after DSS administration (Fig. 1B). The reduction of food intake in Group 5 (0.1% ZER plus 0.1% NIM) was greater at the end of the experiment as compared with Groups 2–4, though statistically it was not significant. The average body weight of all mice was 31.4 ± 2.5 g at the beginning of the experiment, which increased to 34.5 ± 3.2 g in Group 1 after 2 weeks. In contrast, mice in the other four groups (Groups 2–5) lost weight (by 3.5–5.4 g) throughout the experiment, though there were no significant body weight differences between these groups (Fig. 2A).

3.2. Changes in proinflammatory cytokines

The levels of IL-1α in the colonic mucosa of Group 2 increased by 3.5-fold ($P < 0.001$) as compared with Group 1, however, those in Groups 2–5 did not change significantly (Fig. 2B). It was notable that DSS administration led to a dramatic increase in the level of IL-1β (50-fold) in Groups 2–5 when compared with Group 1 (Fig. 2C). Dietary feedings of ZER (Group 3) and a ZER/NIM combination (Group 5) for 2 weeks significantly decreased DSS-induced IL-1β production by 34% ($P < 0.05$) and 49% ($P < 0.01$), respectively, while the suppressive effect

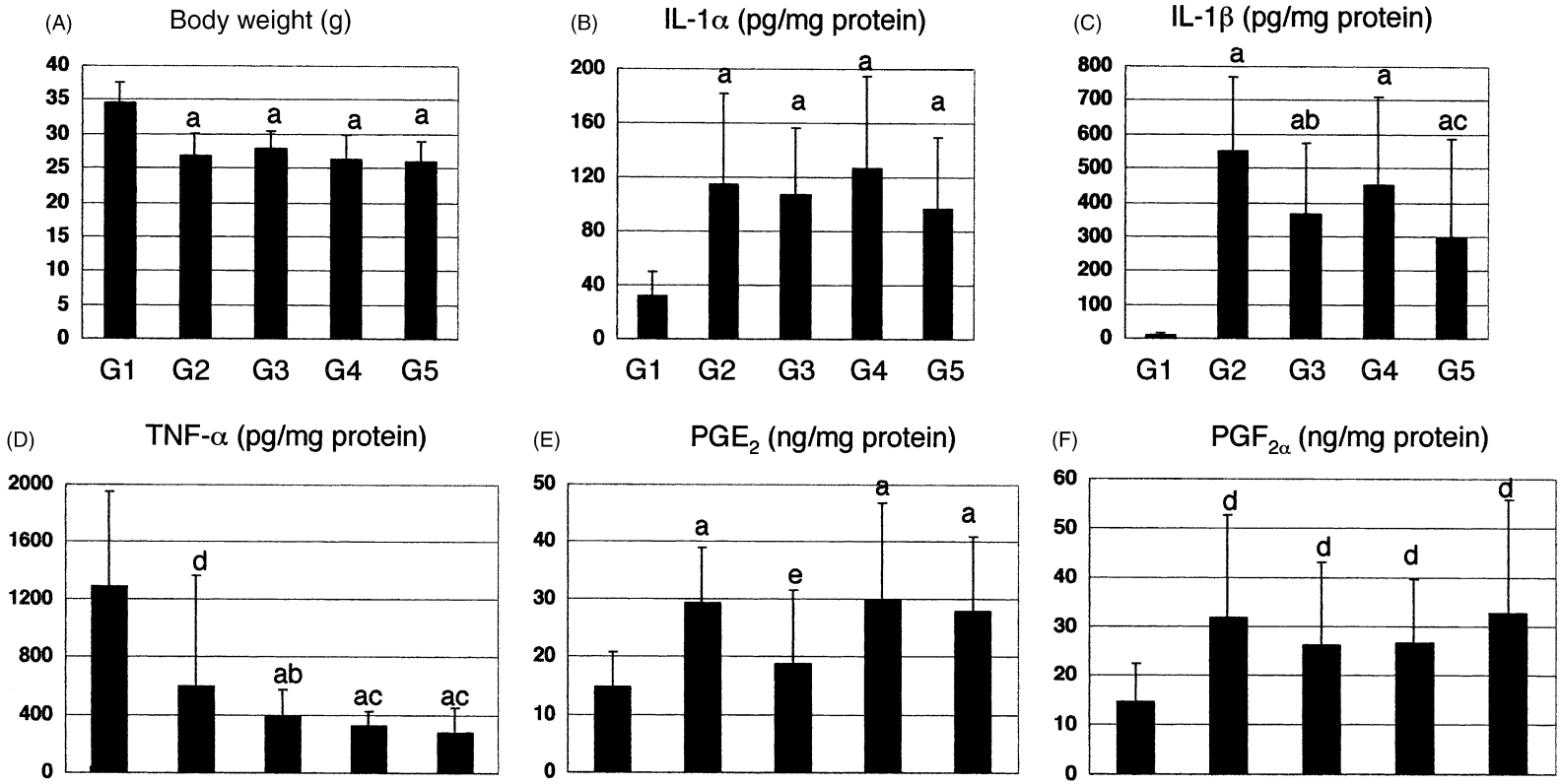

Fig. 2. Body weights, and concentrations of inflammatory cytokines and prostaglandins in colonic mucosa from each group. Panel A, Group 1 (control); panel B, Group 2 (5% DSS), panel C (5% DSS + 0.1% ZER); panel D (5% DSS + 0.1% NIM); and panel E (5% DSS + 0.1% ZER + 0.1% NIM). [a]$P < 0.001$ vs. Group 1, [b]$P < 0.05$ vs. Group 2, [c]$P < 0.01$ vs. Group 2, [d]$P < 0.05$ vs. Group 1, [e]$P < 0.02$ vs. Group 2.

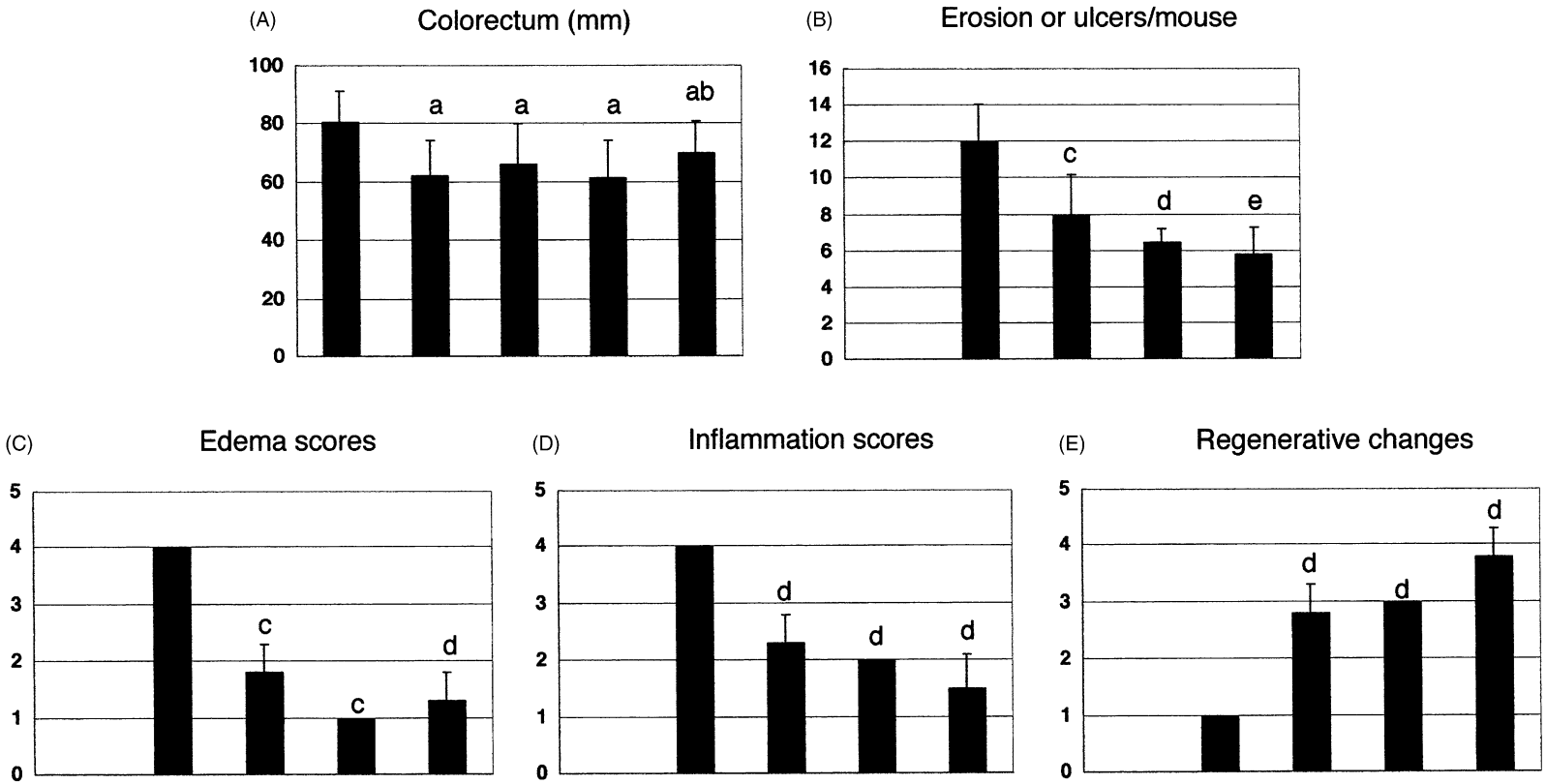

Fig. 3. Colorectum length and histological scores in each group. Panel A, Group 1 (control); panel B, Group 2 (5% DSS), panel C (5% DSS + 0.1% ZER); panel D (5% DSS + 0.1% NIM); and panel E (5% DSS + 0.1% ZER + 0.1% NIM). [a]$P < 0.001$ vs. Group 1, [b]$P < 0.02$ vs. Group 2, [c]$P < 0.01$ vs. Group 2, [d]$P < 0.001$ vs. Group 2, [e]$P < 0.005$ vs. Group 2.

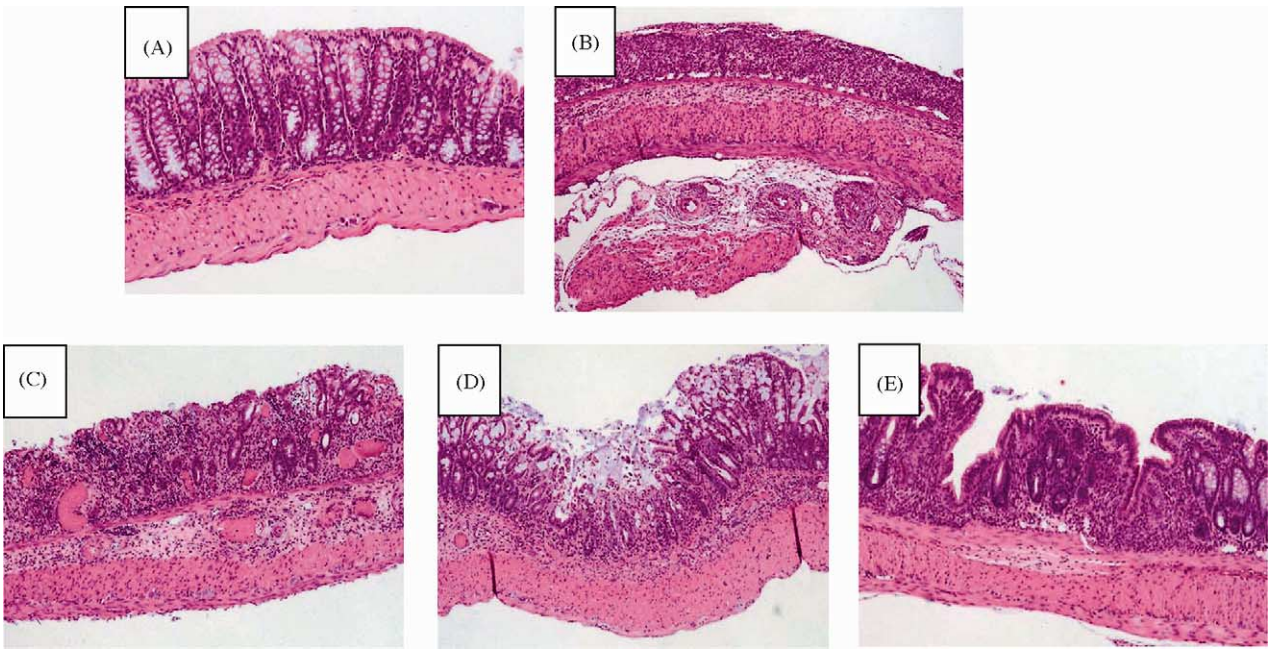

Fig. 4. Hematoxylin and eosin staining of large intestines from each group. Panel A, Group 1 (control); panel B, Group 2 (5% DSS), panel C (5% DSS + 0.1% ZER); panel D (5% DSS + 0.1% NIM); and panel E (5% DSS + 0.1% ZER + 0.1% NIM). Representative pictures are shown. Original magnification, panel A 100×, panels B–E 40×.

of NIM was insignificant. We then quantified TNF-α levels using ELISA and unexpectedly found that DSS treatment attenuated TNF-α production by 46% in Group 2 (Fig. 2D). Administrations of ZER (Group 3), NIM (Group 4), and their combination (Group 5) markedly decreased TNF-α levels by 34, 45, and 53%, respectively, as compared with Group 2.

3.3. Changes in PGs

DSS in drinking water for 1 week resulted in a marked elevation of PGE_2 levels in colonic mucosa by 2-fold ($P < 0.001$) and $PGF_{2\alpha}$ by 2.2-fold ($P < 0.05$) (Fig. 2E and F). On the other hand, ZER dramatically reduced PGE_2 formation by 73%, whereas NIM and the ZER/NIM combination did not. In addition, changes of $PGF_{2\alpha}$ levels among Groups 2–5 were not significant.

3.4. Histological examinations

The occurrence of UC can be histologically confirmed on the basis of erosive and ulcerative lesions, edema formation, and leukocytic infiltration (Fig. 4B). In general, it is known that the length of the large intestine is shortened in mice with colitis. Conversely, the length of the large intestine of the DSS group (62.3 ± 17 mm, $P < 0.001$) was significantly decreased by 23% as compared with that of the control group (80.6 ± 11 mm) (Fig. 3A). Further, the combination of ZER and NIM suppressed colorectum shortening by 42% ($P < 0.02$), while the ZER group showed a tendency for reduction (by 21%), though it was not significant. The mean number of erosive or ulcerative lesions per colon, 12 ± 2.1 in Group 2, were markedly reduced in Groups 3–5 (ZER: by 33%, NIM: by 46%, and ZER/NIM: by 52%). Moreover, edema formation was attenuated by 55, 75, and 68%, respectively, as were the inflammatory indices. Tissue regeneration, confirmed by histological observation, was enhanced in Groups 3–5, and synergistic regeneration by ZER (2.8 ± 0.5 in Group 3) and NIM (3.0 ± 0 in Group 4) was observed in Group 5 (3.8 ± 0.5). Representative histological images from Groups 1 and 2 are shown in Fig. 4.

4. Discussion

In the present study we demonstrated that a food chemical, ZER, has a marked suppressive effect on DSS-induced colitis in mice. This is the first known report showing the suppressive effect of a food phytochemical on DSS-induced colitis, though NIM has recently been reported to reduce DSS-induced superoxide and 8-hydroxy-deoxyguanosine formation in rats [15].

COX-2 expression is one of the key steps in various inflammatory pathogeneses in the digestive tract, such as colitis [9,10], and leads to an accelerated synthesis of PGH_2, and then of PGE_2, $PGF_{2\alpha}$, and PGD_2. It is worth noting that dietary feeding of ZER to mice reduced PGE_2 formation in the colonic mucus membrane (Table 1), which was in accordance with our previous data showing that 4 weeks of ZER feeding dramatically attenuated azoxymethane-induced COX-2 protein expression as well as

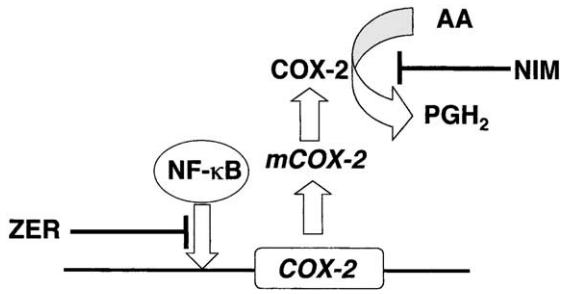

Fig. 5. Distinct modes of action mechanisms of ZER and NIM. While ZER suppressed *de novo* synthesis of COX-2 via attenuation of NF-κB activity, NIM selectively inhibited COX-2 catalytic activity.

PGE_2 formation, as detected by Western blotting and ELISA, respectively [21]. The detailed mechanism by which ZER inhibits PGE_2 formation remains to be elucidated, however, ZER is a prominent inhibitor of COX-2 *de novo* synthesis [17], unlike non-steroidal anti-inflammatory drugs (NSAIDs) that selectively inhibited COX-2 catalytic activity (Fig. 5), but did not affect COX-2 protein synthesis. In addition, we have recently found that ZER suppressed extracellular signal-regulated kinase phosphorylation, thereby inhibiting nuclear factor-kappaB (NF-κB) activity in phorbol ester-treated mouse skin (Murakami A. *et al*., in preparation), suggesting that ZER is able to circumvent inflammatory processes through perturbation of NF-κB transcriptional activity.

The use of compounds with different action mechanisms is currently anticipated to be a reasonable and effective means of disease control [22]. In this context, it should be noted that synergism was seen with respect to the suppression of IL-1β and TNF-α release (Fig. 2C and D), colorectum shortening (Fig. 3A), and histological scores (Fig. 3B and E), as compared with the results of each effect alone.

We have also examined the inhibitory effect of test chemicals on $PGF_{2\alpha}$ production, which has been shown to be involved in human UC [23], though no test chemicals in the present study suppressed $PGF_{2\alpha}$ formation. The reason why NIM did not interfere with PGE_2 and $PGF_{2\alpha}$ formation is uncertain, however, it may be that the dose- and duration-settings of NIM were inappropriate, leading to the unexpected negative results. In contrast, a recent clinical trial demonstrated that COX-2 inhibitors are promising drugs for the regulation of inflammatory bowel diseases, including colitis [24].

A proinflammatory cytokine, IL-1β, has been shown to play some pivotal roles in UC development [25,26]. Further, another intriguing role for the cytokine, autocrine production of IL-1β confers the activity of constitutive NF-κB [27], which is a transcriptional factor that has a major involvement in many aspects of inflammatory events, has recently been discovered. In the present study as well, IL-1β was the most distinct proinflammatory mediator on the basis of its dramatic elevation (50-fold over the control), suggesting that an understanding of the suppressive efficacy of ZER on IL-1β production may be essential to elucidate its anti-inflammatory mechanisms.

In the present study, colonic TNF-α levels in the DSS group were decreased by 54% 1 week after DSS treatment as compared with Group 1. Further, TNF-α levels in Group 2 were consistently lower than those in Group 1 during the first week (data not shown). In contrast, Korenaga *et al*. and other groups have reported that TNF production is enhanced by DSS administration [28]. Accordingly, an increased expression of TNF-α messenger RNA in the intestinal mucosa of inflammatory bowel disease patients, particularly in those in the inactive phase, has been demonstrated, though controversial data has also been reported, e.g. TNF-α mRNA expression was found unchanged in both UC patients and a healthy population [29], the TNF-α antibody failed to mitigate UC [30], and serum TNF-α levels in UC patients were indistinguishable from healthy controls [31]. Furthermore, in acute but not chronic colitis, treatment with the anti-TNF monoclonal antibody led to aggravation [32]. Taken together, these results show that it is premature to conclude that TNF-α is positively involved in the pathogenesis of UC. In any case, it is notable that ZER, NIM, and their combination reduced TNF-α formation as compared with the DSS group, which is partly consistent with our previous results from cell culture experiments [33].

It is well known that oxidative stress is involved in the development of colitis [4] and its mechanism has been suggested to be destruction of the oxidative defense system, such as depletion of an endogenous antioxidant, glutathione [28]. On the other hand, it is of paramount importance to note that neutrophil-infiltration and activation is associated with enhanced oxidative and nitrosative injuries [34]. In particular, Krieglstein *et al*. recently reported a very interesting finding that iNOS, rather than NADPH oxidase, is a key enzyme that triggers colitis pathogenesis, as shown by an extensive study that used a series of knockout mice [35]. We previously demonstrated that ZER is a prominent inhibitor of iNOS expression in endotoxin-stimulated RAW 264.7 macrophages [17]. Although nitrosative stress status was not assessed in the present study, a potential antinitrosative property of ZER may partly account for its action mechanisms for colitis suppression.

Although we only addressed the preventive efficacy of ZER or NIM in this study, the curative potentials of these chemicals should also be evaluated in the near future because the prognosis of UC is generally difficult. In addition, we can not role out the possibility that these chemicals chemically interacted with DSS, although they are stable in the experimental diet at least 1 week at room temperature (data not shown).

In conclusion, we found a higher suppressive effect of ZER than NIM on DSS-induced colitis in mice, which is one of the animal models for intestinal inflammation.

These and our previous results strongly suggest the use of ZER alone and in combination with selective COX-2 inhibitors for controlling inflammatory bowel diseases.

Acknowledgments

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References

[1] Tanaka T, Kohno H, Murakami M, Shimada R, Kagami S. Colitis-related rat colon carcinogenesis induced by 1-hydroxyanthraquinone and methylazoxymethanol acetate (Review). Oncol Rep 2000;7: 501–8.

[2] Okayasu I, Hatakeyama S, Yamada M, Ohkusa T, Inagaki Y, Nakaya R. A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice. Gastroenterology 1990;98: 694–702.

[3] Blackburn AC, Doe WF, Buffinton GD. Protein carbonyl formation on mucosal proteins *in vitro* and in dextran sulfate-induced colitis. Free Radic Biol Med 1999;27:262–70.

[4] D'Odorico A, Bortolan S, Cardin R, D'Inca R, Martines D, Ferronato A, Sturniolo GC. Reduced plasma antioxidant concentrations and increased oxidative DNA damage in inflammatory bowel disease. Scand J Gastroenterol 2001;36:1289–94.

[5] Kankuri E, Asmawi MZ, Korpela R, Vapaatalo H, Moilanen E. Induction of iNOS in a rat model of acute colitis. Inflammation 1999;23:141–52.

[6] Obermeier F, Kojouharoff G, Hans W, Scholmerich J, Gross V, Falk W. Interferon-gamma (IFN-gamma)- and tumour necrosis factor (TNF)-induced nitric oxide as toxic effector molecule in chronic dextran sulphate sodium (DSS)-induced colitis in mice. Clin Exp Immunol 1999;116:238–45.

[7] Clapper ML, Adrian RH, Pfeiffer GR, Kido K, Everley L, Cooper HS, Murthy S. Depletion of colonic detoxication enzyme activity in mice with dextran sulphate sodium-induced colitis. Aliment Pharmacol Ther 1999;13:389–96.

[8] Arai Y, Takanashi H, Kitagawa H, Okayasu I. Involvement of interleukin-1 in the development of ulcerative colitis induced by dextran sulfate sodium in mice. Cytokine 1998;10:890–6.

[9] Agoff SN, Brentnall TA, Crispin DA, Taylor SL, Raaka S, Haggitt RC, Reed MW, Afonina IA, Rabinovitch PS, Stevens AC, Feng Z, Bronner MP. The role of cyclooxygenase 2 in ulcerative colitis-associated neoplasia. Am J Pathol 2000;157:737–45.

[10] Singer II, Kawka DW, Schloemann S, Tessner T, Riehl T, Stenson WF. Cyclooxygenase 2 is induced in colonic epithelial cells in inflammatory bowel disease. Gastroenterology 1998;115:297–306.

[11] Yoshimi N, Ino N, Suzui M, Tanaka T, Nakashima S, Nakamura M, Nozawa Y, Mori H. The mRNA overexpression of inflammatory enzymes, phospholipase A2 and cyclooxygenase, in the large bowel mucosa and neoplasms of F344 rats treated with naturally occurring carcinogen, 1-hydroxyanthraquinone. Cancer Lett 1995;97: 75–82.

[12] Raab Y, Sundberg C, Hallgren R, Knutson L, Gerdin B. Mucosal synthesis and release of prostaglandin E2 from activated eosinophils and macrophages in ulcerative colitis. Am J Gastroenterol 1995;90: 614–20.

[13] Tanaka T, Kohno H, Yoshitani S, Takashima S, Okumura A, Murakami A, Hosokawa M. Ligands for peroxisome proliferator-activated receptors alpha and gamma inhibit chemically induced colitis and formation of aberrant crypt foci in rats. Cancer Res 2001;61: 2424–8.

[14] Kankuri E, Vaali K, Korpela R, Paakkari I, Vapaatalo H, Moilanen E. Effects of a COX-2 preferential agent nimesulide on TNBS-induced acute inflammation in the gut. Inflammation 2001;25:301–10.

[15] Tardieu D, Jaeg JP, Deloly A, Corpet DE, Cadet J, Petit CR. The COX-2 inhibitor nimesulide suppresses superoxide and 8-hydroxy-deoxyguanosine formation, and stimulates apoptosis in mucosa during early colonic inflammation in rats. Carcinogenesis 2000;21: 973–6.

[16] Lesch CA, Kraus ER, Sanchez B, Gilbertsen R, Guglietta A. Lack of beneficial effect of COX-2 inhibitors in an experimental model of colitis. Methods Find Exp Clin Pharmacol 1999;21:99–104.

[17] Murakami A, Takahashi D, Kinoshita T, Koshimizu K, Kim HW, Yoshihiro A, Nakamura Y, Jiwajinda S, Terao J, Ohigashi H. Zerumbone, a Southeast Asian ginger sesquiterpene, markedly suppresses free radical generation, proinflammatory protein production, and cancer cell proliferation accompanied by apoptosis: the alpha,beta-unsaturated carbonyl group is a prerequisite. Carcinogenesis 2002;23: 795–802.

[18] Murakami A, Takahashi M, Jiwajinda S, Koshimizu K, Ohigashi H. Identification of zerumbone in *Zingiber zerumbet* Smith as a potent inhibitor of 12-*O*-tetradecanoylphorbol-13-acetate-induced Epstein-Barr virus activation. Biosci Biotechnol Biochem 1999;63: 1811–2.

[19] Kitajima S, Takuma S, Morimoto M. Changes in colonic mucosal permeability in mouse colitis induced with dextran sulfate sodium. Exp Anim 1999;48:137–43.

[20] Cooper HS, Murthy SN, Shah RS, Sedergran DJ. Clinicopathologic study of dextran sulfate sodium experimental murine colitis. Lab Invest 1993;69:238–49.

[21] Tanaka T, Shimizu M, Kohno H, Yoshitani S, Tsukio Y, Murakami A, Safitri R, Takahashi D, Yamamoto K, Koshimizu K, Ohigashi H, Mori H. Chemoprevention of azoxymethane-induced rat aberrant crypt foci by dietary zerumbone isolated from *Zingiber zerumbet*. Life Sci 2001;69:1935–45.

[22] Torrance CJ, Jackson PE, Montgomery E, Kinzler KW, Vogelstein B, Wissner A, Nunes M, Frost P, Discafani CM. Combinatorial chemoprevention of intestinal neoplasia. Nat Med 2000;6:1024–8.

[23] Lauritsen K, Laursen LS, Bukhave K, Rask-Madsen J. *In vivo* profiles of eicosanoids in ulcerative colitis, Crohn's colitis, and *Clostridium difficile* colitis. Gastroenterology 1988;95:11–7.

[24] Mahadevan U, Loftus Jr EV, Tremaine WJ, Sandborn WJ. Safety of selective cyclooxygenase-2 inhibitors in inflammatory bowel disease. Am J Gastroenterol 2002;97:910–4.

[25] Arai Y, Takanashi H, Kitagawa H, Okayasu I. Involvement of interleukin-1 in the development of ulcerative colitis induced by dextran sulfate sodium in mice. Cytokine 1998;10:890–6.

[26] Fukushima K, West G, Fiocchi C. Adequacy of mucosal biopsies for evaluation of intestinal cytokine-specific mRNA. Comparative study of RT-PCR in biopsies and isolated cells from normal and inflamed intestine. Dig Dis Sci 1995;40:1498–505.

[27] Arlt A, Vorndamm J, Muerkoster S, Yu H, Schmidt WE, Folsch UR, Schafer H. Autocrine production of interleukin 1beta confers constitutive nuclear factor kappaB activity and chemoresistance in pancreatic carcinoma cell lines. Cancer Res 2002;62:910–6.

[28] Korenaga D, Takesue F, Kido K, Yasuda M, Inutsuka S, Honda M, Nagahama S. Impaired antioxidant defense system of colonic tissue and cancer development in dextran sulfate sodium-induced colitis in mice. J Surg Res 2002;102:144–9.

[29] Dionne S, Hiscott J, D'Agata I, Duhaime A, Seidman EG. Quantitative PCR analysis of TNF-alpha and IL-1 beta mRNA levels in pediatric IBD mucosal biopsies. Dig Dis Sci 1997;42:1557–66.

[30] Olson AD, DelBuono EA, Bitar KN, Remick DG. Antiserum to tumor necrosis factor and failure to prevent murine colitis. J Pediatr Gastroenterol Nutr 1995;21:410–8.

[31] Gotteland M, Lopez M, Munoz C, Saez R, Altshiller H, Llorens P, Brunser O. Local and systemic liberation of proinflammatory cytokines in ulcerative colitis. Dig Dis Sci 1999;44:830–5.

[32] Kojouharoff G, Hans W, Obermeier F, Mannel DN, Andus T, Scholmerich J, Gross V, Falk W. Neutralization of tumour necrosis factor (TNF) but not of IL-1 reduces inflammation in chronic dextran sulphate sodium-induced colitis in mice. Clin Exp Immunol 1997; 107:353–8.

[33] Nieto N, Torres MI, Fernandez MI, Giron MD, Rios A, Suarez MD, Gil A. Experimental ulcerative colitis impairs antioxidant defense system in rat intestine. Dig Dis Sci 2000;45:1820–7.

[34] Yamada T, Grisham MB. Role of neutrophil-derived oxidants in the pathogenesis of intestinal inflammation. Klin Wochenschr 1991;69: 988–94.

[35] Krieglstein CF, Cerwinka WH, Laroux FS, Salter JW, Russell JM, Schuermann G, Grisham MB, Ross CR, Granger DN. Regulation of murine intestinal inflammation by reactive metabolites of oxygen and nitrogen: divergent roles of superoxide and nitric oxide. J Exp Med 2001;194:1207–18.