INTRODUCTION

The first report on the use of dextran sulfate sodium (DSS) dates back in the year 1985, when Ohkusa et al. published their investigation on DSS-induced colitis in hamsters (1), after which DSS colitis was induced in mice (2). Today, there are quite numerous studies using DSS-induced colitis model to investigate pathogenesis of colitis. Different factors affected the development of colitis in this DSS administration, the concentration, the duration, and frequency of DSS administration, by which the animals may develop acute or chronic colitis or even colitis-induced dysplastic lesions depending on administration scheme (3). Furthermore, mice show different susceptibilities and responsiveness to DSS-induced colitis dependent on not only concentration, molecular weight, duration of exposure of DSS, but also genetic and microbiological factors of animal (4).

As core pathogenesis of DSS-induced colitis, DSS is toxic to colonic epithelial cells and causes defects in the epithelial barrier integrity, whereby increasing the colonic mucosal permeability to allow permeation of large molecules such as DSS. Though the mechanism of how DSS passes through the mucosal epithelial cells transcellularly or paracellularly remains uncertain, loss of one of the components of tight junction protein and increased expression of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1 β (IL-1 β), interferon-γ (IFN-γ), interleukin-10 (IL-10), and IL-12 accompanied with histological changes including mucin depletion, epithelial degeneration, necrosis leading to disappearance of epithelial cells, neutrophils infiltration of lamina propria and submucosa, cryptitis, crypt abscesses, and phlegmonous inflammation in mucosa and submucosa are intervened (5-9).

HOST NUCLEAR FACTOR ERYTHROID 2-RELATED FACTOR-2 DEFENSE SYSTEM DETERMINES THE OUTCOME OF DEXTRAN SULFATE SODIUM-INDUCED COLITIS IN MICE

Administration of dextran sulfate sodium (DSS) in drinking water led to significant bout of colitis simulating ulcerative colitis of human. However, colitis usually developed 5 – 7 days after DSS administration. Therefore, we hypothesized host defense system might protect colitis up to 5 days of DSS administration. 2.5% DSS-induced colitis were administered to C57BL/6 mice and sequential measurements of pathology, cyclooxygenase-2 (COX-2), nuclear factor-xB (NF-xB), heme oxygenase-1 (HO-1), NADPH quinone oxidoreductase-1 (NQO1), γ-glutamylcysteine synthetase (γ-GCS), nuclear factor erythroid 2-related factor-2 (Nrf2), and keap1 were done at 2, 6, 12, 24, 48, 96, 120, and 168 hour of DSS administration, respectively. DSS-induced colitis was repeated in either COX-2−/− or Nrf2−/− mice. On serial pathological analysis, significant colitis was noted after 120 h of DSS administration, during which both activations of COX-2/NF-xB and HO-1/Nrf2 were noted. Nrf2 activations after keap1 inactivation led to significant increases in HO-1 after 168 hours of DSS administration, when NF-xB nuclear translocation was noted. Significantly attenuated colitis was noted in DSS-challenged COX-2−/− mice, in which the levels of HO-1 were significantly decreased compared to DSS-challenged WT littermates (p < 0.01), while the levels of NQO1 were significantly increased. On DSS administration to Nrf2−/− mice, colitis was significantly aggravated (p < 0.01), in which the expressions of COX-2 as well as expressions of HO-1 and γ-GCS were significantly increased (p < 0.01). Reciprocal activations of inflammatory and antioxidative defense signaling after DSS administration might be prerequisite to make intestinal homeostasis and host defense Nrf2 system can determine colitis.

Key words: experimental, colitis, nuclear factor erythroid 2-related factor-2, heme oxygenase-1, cyclooxygenase-2, keap1, host defense system, intestinal permeability
DSS, before which no significant changes in either gross finding or clinical symptoms were noted. In previous publication by Kim et al. (10), we reported the preemptive administration of oligonol, oligomerized polyphenol extracted from fruit lychee, significantly afforded either the attenuation of DSS-induced colitis or lowered relapse of DSS-induced colitis. Therefore, as a plausible explanation about why significant pathology does not appear before 4 – 5 days of DSS administration, we hypothesized that host defense Nrf2 system might contribute to these rescuing actions from toxic DSS. Since the current strategy for maintenance therapy, anti-inflammatory drug such as sulfasalazine or mesalazine is prescribed, we aim to put stress the necessity of intervention of agent increasing host defense system in the maintenance therapy of inflammatory bowel diseases (IBD).

In this study, we have checked and correlated with DSS-induced pathologies in serial measurements of pathology, changes of cyclooxygenase-2 (COX-2)/nuclear factor-xB (NF-xB), and heme oxygenase-1 (HO-1)/nuclear factor erythroid 2-related factor 2 (Nrf2) in 2.5% DSS-administered colitis model at 2, 6, 12, 24, 48, 96, 120, and 168 h of DSS administration, respectively. Also, DSS-induced colitis was repeated in either COX-2 or Nrf2 knockout (KO) mice with the measurement of COX-2 and HO-1/NADPH quinone oxidoreductase-1 (NQO1)/γ-glutamylcysteine synthetase (γ-GCS)/Nrf2 with the conclusion that host defense Nrf2 system can determine colitis.

MATERIALS AND METHODS

Animals

Animals were handled in an accredited animal facility in accordance with Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International) guidelines under the facility named CACU (The Center of Animal Care and Use) of CHA University Laboratory Animal Research Center after Institutional Review Board approval, briefly, never more than 4 animals per cage and under the best housing conditions.

Reagents

The following materials were obtained from commercial sources: all chemical reagents from Sigma (St. Louis, MO) and antibodies for Western blotting were purchased as follows: β-actin, NF-xB p65, lamin B, HO-1 antibodies from Santa Cruz Biotechnology (Santa Cruz, CA), iNOS antibody from BD Biosciences (San Jose, CA), COX-2 antibody from Thermo Scientific (Seoul, Korea), NQO-1 antibody from Abcam (Cambridge, MA). Horseradish peroxidase-conjugated anti-rat/rabbit/mouse IgG was purchased from Thermo Scientific Pierce (Rockford, IL).

Dextran sulfate sodium administration to induce colitis

As for DSS-induced colitis, germ-free male C57BL/6 mice (5 weeks of age, Orient Bio, Seongnam, Korea) were used for the experiments. A total of 80 wild type mice were administered with 2.5% DSS in drinking water. The mice were maintained up to 7 days of DSS administration, during which 8 mice per group were sacrificed after 2 h of 2.5% DSS administration to 168 h, 2, 6, 12, 24, 48, 72, 96, 120, and 168 h, respectively. Colon were resected and homogenated for the following experiments, pathology, Western blot, EMSA, and RT-PCR. Also, COX-2−/−, ARE hPAP−/−, ARE hPAP+/−, and

Nrf2−/− mice were all purchased from Jackson lab and breded up to 12 weeks according to instruction with genotyping. Total 80 KO mice (20 per each gene KO) were used in this experiment.

Assessment of colonic damage

Animal body weight, the presence of gross blood in the feces, and stool consistency were recorded daily for each rat by an observer unaware of the treatment. None of the mice were died in all the groups. Once mice were sacrificed, their colons were immediately removed and rinsed with ice-cold phosphate-buffered saline. The excised colonic segments were placed on an ice-cold plate, cleaned of fat and mesentery, and blotted on filter paper. Each specimen was weighed and its length measured under a constant load (2 g). The colon was longitudinally opened, and a cross section from the distal diseased area was immediately fixed in 3.7% formaldehyde and embedded in paraffin for histological analysis. Afterward, it was sectioned into different longitudinal fragments to be used for biochemical determination and Western blotting.

Histopathological examinations

The paraffin sections were stained with hematoxylin and eosin (H&E) or saved for immunohistochemical staining. Pathologic index was graded according to criteria (38). Pathologic data and slides were blindly reviewed by two independent gastrointestinal specialists (Kim KJ and Hahm K-B).

Western blot analysis

The colon tissues were homogenized with ice-cold cell lysis buffer (Cell Signaling Technology, Danvers, MA) containing 1 mM phenylmethylsulfonyl fluoride (PMSE). After 20 min of incubation, samples were centrifuged at 12,000 g for 15 min. Supernatants were then collected. Total protein-equivalents for each sample were separated by SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membranes, which were incubated with which were incubated with appropriate antibodies and then visualized using West-zol Plus (Intron biotechnology, Seongnam, Korea).

Electrophoretic mobility shift assay (EMSA)

Nuclear proteins were incubated with a solution consisting of 20 mM HEPES, 4 mM MgCl2, 50 mM CaCl2, 1 mM EDTA, 1 mM DTT, and 4% glycerol. The mixtures were loaded onto a non-denaturing 6% polyacrylamide gel with 0.25 × TBE electrophoresis buffer. After electrophoresis, gels were dried and exposed to the radiography film for 24 h at –70ºC with intensifying screens. Each anti-p50 and -p65 antibody (Santa Cruz, CA) was mixed with the NF-xB probe at the start of the 30 min incubation.

Statistical analysis

The data are presented as means ± standard deviations (SD). The data were analyzed by ONE-WAY ANOVA, and the statistical significance between groups was determined by Student’s t-test. Statistical significance was accepted when p < 0.05. The survival curves between the groups were compared using log-rank test.
Fig. 1. 2.5% DSS-induced colitis at different time point (A) Representative pathology according to times after 2.5% DSS administration, ×40 magnification except two boxed figures of 120 and 168 h, which is ×100 magnification. (B) COX-2 expression according to different times after 2.5% DSS administration. (C) Cox-2 mRNA expression according to different times after 2.5% DSS administration by qRT-PCR, *denoted statistical significance (p < 0.05 versus 0 h), n = 8/each time. (D) EMSA for NF-κB. We used 32p-labeled NF-xB probe for EMSA. Experiments were done in triplicate. (E) Western blot for IκB-α in control and 2.5% DSS administration (168h). Statistical difference in mean expression of IκB-α was noted between vehicle and 2.5% DSS administration, 168 h.
RESULTS

Significant ulcerative colitis after 120 hours of 2.5% dextran sulfate sodium administration, whereas no significant pathology before 120 h in spite of dextran sulfate sodium administration

2.5% DSS administration to C57BL/6 usually led to mild to moderate degree of colitis. On gross observation after 2.5% DSS in drinking water, diarrhea and hematochexia usually developed after 5 – 7 days. In this condition, when we sacrificed animal, as seen in Fig. 1A, overt pathology was noted after 120 h after DSS administration including colon ulcer, severe inflammatory cell infiltrations, and some hemorrhages. These findings were further aggravated after 168 h of 2.5% DSS administration. On serial measurement of body weight changes, significant loss of weight (mean decreases of 10%) was also noted from 120 h of DSS administration. Before 120 h, only very mild inflammatory cell infiltrations without any mucosal damages were noted around 72 – 96 h. Using mucosal homogenates, the expressions of COX-2 mRNA and proteins were traced according to different time. As seen in Fig. 1B, the expressions of COX-2 mRNA and protein COX-2 were significantly increased after 120 h (p < 0.05, Fig. 1B and 1C). Since NF-κB is transcription factor responsible for COX-2 induction, we measured NF-κB-DNA binding with enzyme mobility shift assay (EMSA). As seen in Fig. 1D, significantly increasing NF-κB-DNA binding was noted after 72 h. IκB-α reflects NF-κB activation, by which the mean expression of IκB-α was significantly decreased after 168 h of 2.5% DSS administration (Fig. 1E).

Diverse expressions of host defensive proteins were also co-expressed after 2.5% dextran sulfate sodium administration

2.5% DSS administration to C57BL/6 usually led to moderate to severe degree of colitis only after 120 h. Under the hypothesis host defensive response might operate in conjunction with increasing COX-2 after toxic DSS administration, we traced the expressions of HO-1, NQO-1, γ-GCS, and MnSOD in different times after 2.5% DSS administration. As seen in Fig. 2A, the peak expressions of each cytoprotective protein were differed according to pathology. In detail, regarding HO-1, the expressions of HO-1 show two peaks, HO-1 was increasing up to 6 h, but disappeared after 12 h, and then reappeared after 48 h and continued. NQO-1 was increased only around 24 h spatially, but γ-GCS increased after 120 h. However, no significant changes of MnSOD were noted. Each adaptive cytoprotective gene was revalidated with qRT-PCR, of which findings were quite compatible with the findings shown with Western blotting (Fig. 2B-2D). In summary, the expressions of cytoprotective defensive protein were differed according to times of DSS exposure, HO-1 and NQO-1 before overt colon pathology, but γ-GCS and HO-1 coincide with colon pathology (Fig. 2A). Using homogenates, we performed EMSA with Nrf2 because Nrf2 is a transcription factor responsible for these adaptive antioxidative cytoprotective proteins (Fig. 2E).
As expected, the appearance of ulcerative colitis with 2.5% DSS administration at 168 h was significantly associated with increased COX-2 (p < 0.005, Fig. 3A), inducible nitric oxide synthase (iNOS) (p < 0.001, Fig. 3B), Jun-N-terminal kinase (JNK) activation (p < 0.005, Fig. 3C), and protein kinase B (AKT) activation (p < 0.001, Fig. 3D). Taken together with findings from Fig. 1, DSS induced colitis through the activation of inflammatory mediators.

Heme oxygenase-1, nuclear factor erythroid 2-related factor-2, keap1 changes at 168 hour after 2.5% dextran sulfate sodium administration

As expected, the appearance of ulcerative colitis with 2.5% DSS administration at 168 h was significantly associated with increased HO-1 (p < 0.005, Fig. 4A), Nrf2 (p < 0.001, Fig. 4B), and keap1 inactivation (p < 0.05, Fig. 4C). Since Nrf2 is activated with disulfide inactivation of keap1, successfully we confirmed that co-inciding changes of Nrf2 increases and keap1 decreases were noted at colon administered with 2.5% DSS at 168 h. These findings suggested that DSS administration stimulated both inflammatory response as well as host defense Nrf2 system. Compounding the findings from Fig. 3 and Fig. 4, overwhelming host defense system might be the explanation why no significant pathology before 5 days of 2.5% DSS administration.

Mitigated colitis in cyclooxygenase-2 knockout mice with attenuated heme oxygenase-1 expression

In order to measure the intervening action of HO-1 against DSS-induced colitis, we repeated 2.5% DSS challenge in COX-2 KO mice. As noted in Fig. 4A, colon length, reflecting the severity of DSS-induced colitis, was significantly shortened with DSS administration in WT littermate (p < 0.01), but their colon length was significantly preserved in COX-2 KO mice (p < 0.01). In this condition, when we
Fig. 3. Comparison of COX-2, iNOS, JNK, and Akt between control and 2.5% DSS administration (168 h), n = 8/Group (A) COX-2 (B) iNOS (C) p-JNK (D) p-AKT.
measured HO-1, the expressions of HO-1 in COX-2 KO mice challenged with DSS were significantly decreased compared to WT littermates (p < 0.01, Fig. 5B and 5C). As noted before, HO-1 was significantly increased in WT challenged with DSS. Nqo1 mRNA was significantly increased in COX-2 KO mice administered with 2.5% DSS. Therefore, we inferred attenuated HO-1 defense system explain the increased susceptibility to 2.5% DSS in COX-2 KO mice. Interestingly, in spite of attenuated HO-1 expression in COX-2 KO, another host defense system, NQO1, was significantly induced in COX-2 KO mice after DSS administration to compensate attenuated HO-1 (p < 0.01, Fig. 5D).

Fig. 4. Comparison of HO-1, Nrf2, and keap1 between control and 2.5% DSS administration (168 h), n = 8/Group (A) HO-1 (B) Nrf2 (C) Keap1.
Accentuated colitis in nuclear factor erythroid 2-related factor-2 knockout mice

Stimulated with ameliorated colitis in COX-2+/- mice, we further hypothesized that Nrf KO mice might show increased susceptibility to DSS colitis. As seen in Fig. 5A, severe colitis was induced in Nrf2-/- mice upon exposure to 2.5% DSS as evidenced that colon length was significantly decreased in Nrf2-/- mice (p < 0.01). In this condition, when we measured human PAP activity, 2.5% DSS administration still increased hPAP activity in ARE hPAP-/-/+ mice, and further evaluate the status of COX-2 and phase 2 antioxidative enzymes including NQO-1, γ-GCS, and HO-1, we compared the expressions of these genes according to Nrf2 status before and after 2.5% DSS administration. As seen in Fig. 5C, Nrf2-/- mice showed increased expressions of COX-2 even before DSS administration and their expressions were further increased in Nrf2-/- with 2.5% DDS administration (p < 0.01). The expressions of phase 2 antioxidative enzyme gene including γ-GCS and HO-1 were significantly decreased in Nrf2-/- mice both before and after 2/5% DDS administration. Interestingly, irrespective of Nrf2-/- mice and WT mice, qPCR for Cox-2 and Ho-1 mRNA showed significant increases in Nrf2-/- mice (Fig. 6D and 6E), signifying that both inflammatory and anti-inflammatory status is induced with DSS administration, but host defense system might determine the outcomes of DSS-induced colitis because these were induced even in the absence of Nrf2.

DISCUSSION

In this study, under the hypothesis that host adoptive phase 2 response through Nrf2 activation might determine the outcomes of DSS administration, we had checked the phase 2 detoxifying and antioxidative enzyme responses after DSS administration and documented the difference in DSS damages according to COX-2 and Nrf2 status using COX-2 -/– and Nrf2 –/– mice. As results, the host defense Nrf2 system contributed to maintain intestinal homeostasis, after which we concluded that the enhancement of host defense system should be considered in addition to current strategy of anti-inflammatory agents as maintenance therapy of IBD. As shown in Fig. 1A, depletion in host Nrf2 defense system led to overt manifestation of DSS-induced colitis after 120 h. As shown in additional experiments using COX-2 –/– and Nrf2 –/– mice, the effort to induce host defense Nrf2 system continued even in the absence of damaging COX-2 expression and in the absence of Nrf2 system. As seen in Fig. 7, DSS-induced colitis is associated with the up-regulation of different cytokines including...
chemokines, nitric oxide, and iNOS, and cytokine profiles in DSS colitis correlates with clinical and histological parameters as well as barrier properties. As much as inflammatory cytokines, difference in host adoptive response might affect the susceptibility to DSS as exemplified with the phase 2 adoptive antioxidative and anti-mutagenic reaction.

Animal models of intestinal inflammation are indispensable for our understanding of the pathogenesis of IBD, for which 2, 4,
6-trinitro benzene sulfonic acid (TNBS)-, oxazolone-, and DSS-induced colitis are the most widely used chemically induced models of intestinal inflammation as well as colitis-associated cancer (11-14). In the former two models, colitis is induced by intrarectal administration of the covalently reactive reagents TNBS/oxazolone inducing a T-cell-mediated response against hapten-modified autologous proteins and for DSS model, mice are subjected several days to drinking water supplemented with DSS, which seems to be directly toxic to colonic epithelial cells of the basal crypts (14). DSS-induced colitis model, originally reported by Okayasu et al. (2) has been used to investigate the role of leukocytes in the development of colitis. Oral administration of 2–5% DSS in drinking water for one or more weeks can induce not only acute, but also chronic colitis (3). One cycle of 2–5% DSS administration for 5–7 days, followed by regular water, results in extensive injury with complete crypt depletion and relatively slow regeneration of colonic epithelium. This regeneration is much slower than in other acute injury models, which use toxic substances such as acetic acid and ethanol. Depletion of goblet cells is one of the most reliable histological characteristics of ulcerative colitis led to a major symptom of bloody diarrhea containing a large amount of mucus. Scanning electron microscope observations of the colonic mucosal surface after DSS showed a complete subversion of its architecture, characterized by dilations of gland crypt openings, dropout of goblet cells, and inhomogeneous distribution or lack of microvilli. Relevant with molecular changes relevant to these pathologies, increased levels of IL-1β, TNF-α, and platelet activating factor (PAF), whereas cytoprotective PGE2 and NO are markedly decreased (15).

The clinical features of this model include weight loss, loose stools, diarrhea, and brisk rectal bleeding. Pathological analysis typically reveals extensive crypt and epithelial cell damage, significant infiltration of granulocytes and mononuclear immune cells, and submucosal edema, often accompanied with severe and extensive ulceration. Histopathology, by H&E staining (Fig. 1A), is scored based on three parameters, severity of inflammation as mild, moderate, severe, extent of inflammation as mucosal, submucosal, and transmural, and crypt damage as basal one-third damaged, basal two-thirds damaged, crypt lost but surface epithelium present, crypt, and surface epithelium lost. Although the earliest change of acute DSS-induced colitis is a progressive disruption of colonic crypts, macrophages and CD4+ T cells are more prominent in areas of wound healing in the basal portions of the lamina propria. These CD4+ T cells secrete increased levels of IFN-γ and IL-4, which suggests that chronic immune activation mediated by both Th1 and Th2 cells play a pathogenic role in chronic DSS-induced colitis.

During DSS-induced colitis, as noted in Fig. 1A, the fact that overt mucosal damages were seen after 120 hours of DSS administration stimulates us to investigate the molecular changes implicated in either damaging or defensive aspect of colitis. As noted in Fig. 1A of our experiment, the earliest histologic changes which predated colitis was loss of the basal one-third of the crypt around 72 h to 96 h, which progressed with time to loss of the entire crypt resulting in erosions on 120 h. The earliest changes were very focal and not associated with inflammation, but over inflammation became significant after erosions appeared (5). Though the exact mechanism of DSS colitis is presently unknown, the finding of crypt loss without proceeding or accompanying inflammation suggests that the initial insult is at the level of the epithelial cell, inflammation might be a secondary phenomenon (8). C57BL/6 mice exposed

Fig. 7. Schematic presentation what happens with 2.5% DSS administration during colitis development. 2.5% DSS administration led to both damaging event (COX-2 and iNOS via NF-κB transcriptional activation) and defense system (HO-1, NQO1, γ-GCS via Nrf2 transcriptional activation) in colonocytes. However, the finding that prevailing and accentuated host defense Nrf2 system can led to attenuated colitis led to conclusion that host defense status determines colitis.
to DSS for 5 days developed acute colitis that progressed to severe chronic inflammation (9).

The results we have found significantly suggested the implication of host defense system such as NAD(P)H:quinone oxidoreductase 1 (NQO1), γ-GCS, and HO-1 all transcribed by Nrf2 might determine the outcome of colitis after DSS administration. NQO1 is a key enzyme involved in defense against reactive forms of oxygen, which is expressed ubiquitously in all the tissues (16). Under conditions of oxidative stress, expression of NQO1 is induced, and the resulting increase in oxidoreductase protein provides the cell with multiple layers of protection against environmental insults (17). The catalytic activity of NQO1 is directed towards the complete reduction and detoxication of highly reactive quinones, maintaining the endogenous lipid-soluble antioxidants, and requiring for the stabilisation of p53 protein in response to DNA-damaging stimuli (18). The redox sensitivity of NQO1 transcription occurs through a cis-acting antioxidant response element (ARE) as shown in this experiment, which recruits the positively acting basic leucine zipper (bZIP) transcription factor NF-κB p45-related factor 2 (Nrf2) (19). Under normal constitutive conditions, Nrf2 associates with the cytoskeletal-binding protein Keap1, which regulates the subcellular distribution of the bZIP factor, but oxidative stress inhibits the Nrf2-keap1 interaction, thus promoting nuclear accumulation of the transcription factor and transactivation of NQO1 and other ARE-driven genes (Fig. 6).

Nuclear transcription factors Nrf2 and c-Jun bind to the ARE and activate the gene expression. That is, the free Nrf2 translocates in the nucleus and Nrf2 in the nucleus heterodimerizes with c-Jun and binds to the ARE resulting in the induction of NQO1 and other ARE-regulated genes expression (20). Conclusively, evidence for the importance of the antioxidant functions of NQO1 in combating oxidative stress is provided by demonstrations that induction of NQO1 levels or their knockout are associated with decreased and increased susceptibilities to oxidative stress, respectively.

HO-1, the rate-limiting enzyme in the catabolism of heme, followed by production of biliverdin, free iron and carbon monoxide (CO), acts as an endogenous defensive mechanism to reduce inflammation and tissue injury in various animal intestinal injury models induced by ischemia-reperfusion, indomethacin, lipopolysaccharide-associated sepsis, or DSS (21, 22) and contributes to the resolution of inflammation and has protective effects in several organs against oxidative injury. Especially, in the intestinal tract, HO-1 is shown to be transcriptionally induced in response to inflammation, by which the activation of HO-1 may act as an endogenous defensive mechanism to reduce inflammation and tissue injury in the intestinal tract (23). As a ‘therapeutic funnel’, HO-1 is thought to mediate the beneficial effects attributed to other molecules such as IL-10, iNOS, and PGs, by which blockade of HO-1 activity can result in exacerbation of experimental colitis (24, 25). Interestingly as shown in Fig. 2A, two peaks were shown in the serial expressions of HO-1 after DSS administration, earlier around 6 h and later after 48 h, for which we inferred that Nrf2 transcriptional activation was operative as essential for protection against oxidative and inflammatory injury (26) since keap1-Nrf2 system forms the very major node of cellular and organismal defense against oxidative and inflammatory stress of both exogenous and endogenous origins (27) and stands as an important prophylactic and therapeutic target for inflammatory disease, molecular Swiss army knife (28), concluding that former peak as innate immunity and the later peak as adaptive response.

Nrf2 is a basic leucine zipper redox-sensitive transcriptional factor that plays a central role in antioxidative response element (ARE)-mediated induction of antioxidative and phase-2 detoxifying enzymes and related stress-response proteins (29-32). The 5'-flanking regions of genes encoding these cytoprotective proteins contain a specific consensus sequence termed ARE to which Nrf2 binds. Since during the early phase of inflammation, activation of Nrf2-ARE might inhibit the production or expression of pro-inflammatory mediators including cytokines, chemokines, cell adhesion molecules, matrix metalloproteinases, COX-2 and iNOS, Nrf2 plays a critical role in protecting various tissues against inflammation, whereas Nrf2 deficient mice have an increased susceptibility to DSS-induced colitis as shown by the increased severity of colitis following 1 week of oral administration of only 1% DSS (33-35). As similar noted in our experiment, the increased severity of colitis in Nrf2−/− mice was found to be associated with decreased expression of antioxidant/phase II detoxifying enzymes including HO-1, NQO1, UDP-glucoyltransferase 1A1, and glutathione S-transferase (GST) Mu-1. As noted in the current experiment, looking at proinflammatory mediators/cytokines such as COX-2, iNOS, IL-1β, and TNF-α, these expressions were significantly increased in the colonic tissues of Nrf2−/− mice compared with their wild-type littermates, all suggesting that Nrf2 could play an important role in protecting intestinal integrity through down-regulating proinflammatory cytokines and inducting phase II detoxifying enzymes. Without activation of cytoprotective Nrf2/ARE-regulated genes can suppress inflammatory responses, whereas decreased expression of these genes results in enhanced inflammatory responses to oxidant insults.

Since COX catalyzes the rate-limiting step in PG production, the inducible isoform, COX-2, has been implicated in a variety of inflammatory processes as well as colon carcinogenesis (36, 37). COX-1 protein was expressed at equal levels in colonic epithelial cells of normal and IBD, COX-2 protein was not detected in normal epithelial cells but detected in inflamed colon epithelial cells as well as lamina propria mononuclear cells (38). As seen in Fig. 3 and 4, COX-2−/− mice showed decreased susceptibility to a low-dose of DSS that caused colonic epithelial injury in wild-type mice, in which condition, we also found Nrf2 and HO-1 response was maintained. Since COX-2 inhibitor ameliorated severe colitis, reduced the degree of inflammation through reduction of neutrophil infiltration and IL-1β levels, PGE2, and PGD2 synthesis were significantly reduced in DSS-treated groups. Hence COX-1 and COX-2 share a crucial role in the defense of the intestinal mucosa and that neither isoform is essential in maintaining mucosal homeostasis in the absence of injurious stimuli, host defense Nrf2 system seems to be more important than damaging COX expression.

Though we have focused on the imbalance of damaging and defensive status relevant to colitis, in addition to implication of host defense system, the increase in mucosal permeability occurred before the appearance of the inflammatory process, suggesting that an increase in colonic mucosal permeability, leading to the destruction of mucosal barrier function, may play an important role in the induction of DSS-induced murine colitis. However, many studies also supported the inventing of defense system in maintaining intestinal permeability and integrity, enforcing host defense Nrf system might be pivotal in keeping intestinal homeostasis (39, 40). In our previous publication, oligonol administration significantly affects mitigation of DSS-colitis or decreasing relapse of colitis (10). Therefore, we conclude the cooperating strategy to attenuate damaging factor and to enhance defense system should be considered in the maintenance therapy of IBD.

J.S. Lee and J.M. An contributed equally for current study.

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