Note

Impaired Skin Barrier Function in Mice with Colon Carcinoma Induced by Azoxymethane and Dextran Sodium Sulfate

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We have previously reported that impaired skin barrier function was induced by small intestinal injury in mice. Therefore, we postulated that other intestinal diseases might also influence skin barrier function. In this study, we evaluated the skin barrier function of hairless mice with colon carcinoma that was induced by azoxymethane (AOM) and dextran sodium sulfate (DSS). In mice treated with these drugs, we observed elevated transepidermal water loss and reduced skin hydration levels, compared to those in the control mice. In addition, plasma nitrogen di/trioxide (NO_2^-/NO_3^-) levels were significantly elevated, and expression of type I collagen was significantly reduced in the treated mice, compared to those in control. These results suggest that impaired skin barrier function occurs in mice when colon carcinoma is present.

Key words skin barrier function; azoxymethane; dextran sodium sulfate; type I collagen; colon cancer

The skin's barrier function plays an important role in protecting against the penetration of noxious agents, such as allergens, irritants, and microorganisms, as well as against excessive transepidermal water loss. These various barrier functions are largely mediated by the skin's corneocytes or extracellular matrix components.[1] Therefore, to remain healthy, mammals must maintain homeostasis of their skin's barrier function.

We have previously reported that impaired skin barrier function was induced by small intestinal injury in mice.[2] Based on this finding, we postulated that other intestinal diseases might also influence skin barrier function, as there appears to be a close relationship between the skin and intestine. For example, inflammatory bowel disease involves several cutaneous manifestations, such as erythema nodosum and pyoderma gangrenosum.[3] Therefore, we hypothesized that colon carcinoma (a major intestinal disease) affects the skin's barrier function, and evaluated this hypothesis using a mouse model of carcinogenesis that is induced by azoxymethane (AOM) and dextran sodium sulfate (DSS).

MATERIALS AND METHODS

Animals Seven-week old male hairless mice (Hos:HR-1, SLC, Shizuoka, Japan) were used in this study. The mice were maintained at Suzuka University of Medical Science's animal facility, and all procedures were reviewed and approved by our institutional animal care committee. All mice were housed in plastic cages, with free access to drinking water and a pelleted basal diet, under controlled humidity (50±10%), light (12/12 h light/dark cycle), and temperature (25±2°C).

Study Design The mice were quarantined for the first 7 d, and then randomized according to body weight into the experimental (AOM+DSS) and control groups. AOM (a colonic carcinogen) was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.), and DSS with a molecular weight of 36000–50000 was purchased from MP Biomedicals (Solon, OH, U.S.A.). To induce colitis, DSS was dissolved in water at a concentration of 2% (w/v).

Mice in the AOM+DSS group received a single intraperitoneal injection of AOM (10 mg/kg body weight). Starting at 1 week after the injection, animals received 2% DSS in their drinking water for 1 week, and then no further treatment for 18 weeks, as previously described.[4] Control mice were untreated for the duration of the experiment. All animals were sacrificed at the end of the study (week 20) using pentobarbital.

Isolation of Tissue Samples and Blood Immediately after euthanization, a 1 mL blood sample was collected from each mouse *via* cardiac puncture. Plasma was fractionated from the collected blood samples *via* centrifugation at 10000×***g*** for 10 min at 4°C, and the supernatant was used for the subsequent analyses. Tissue samples were isolated from the dorsal skin and colon, followed by fixation in phosphate buffered saline containing 4% paraformaldehyde.

Staining of the Cryosections Fixed tissue specimens were embedded in Tissue Tek Optimum Cutting Temperature Compound, frozen, and cut into 5 μm thick sections. The sections were then stained with hematoxylin and eosin, according to established procedures, to enable histopathological analysis of the tissue.

Measurement of Plasma Interleukin-6 (IL-6) and Nitrogen Di/Trioxide (NO_2^-/NO_3^-) Concentrations After the treatment, we measured plasma IL-6 concentrations using an enzyme-linked immunosorbent assay kit (R&D Systems, MN, U.S.A.), according to the manufacturer's instructions. The plasma levels of NO_2^-/NO_3^- were also measured for each mouse as a surrogate marker for their inducible nitrous oxide synthase expression, using a biochemical kit for these nitrous oxide radicals (DOJINDO Molecular Technologies Inc., Kumamoto, Japan), according to the manufacturer's instructions.

Measurement of Transepidermal Water Loss (TEWL) and Skin Hydration TEWL and skin hydration levels for the dorsal skin were measured after the treatment, according to previously described methods.[2]

Western Blotting The fixed dorsal skin samples were homogenized in a lysis buffer (Kurabo, Osaka, Japan), which was followed by centrifugation at 8000×***g*** for 10 min. The supernatant from each sample was then isolated and stored at −80°C until analysis. After thawing, equal amounts of protein

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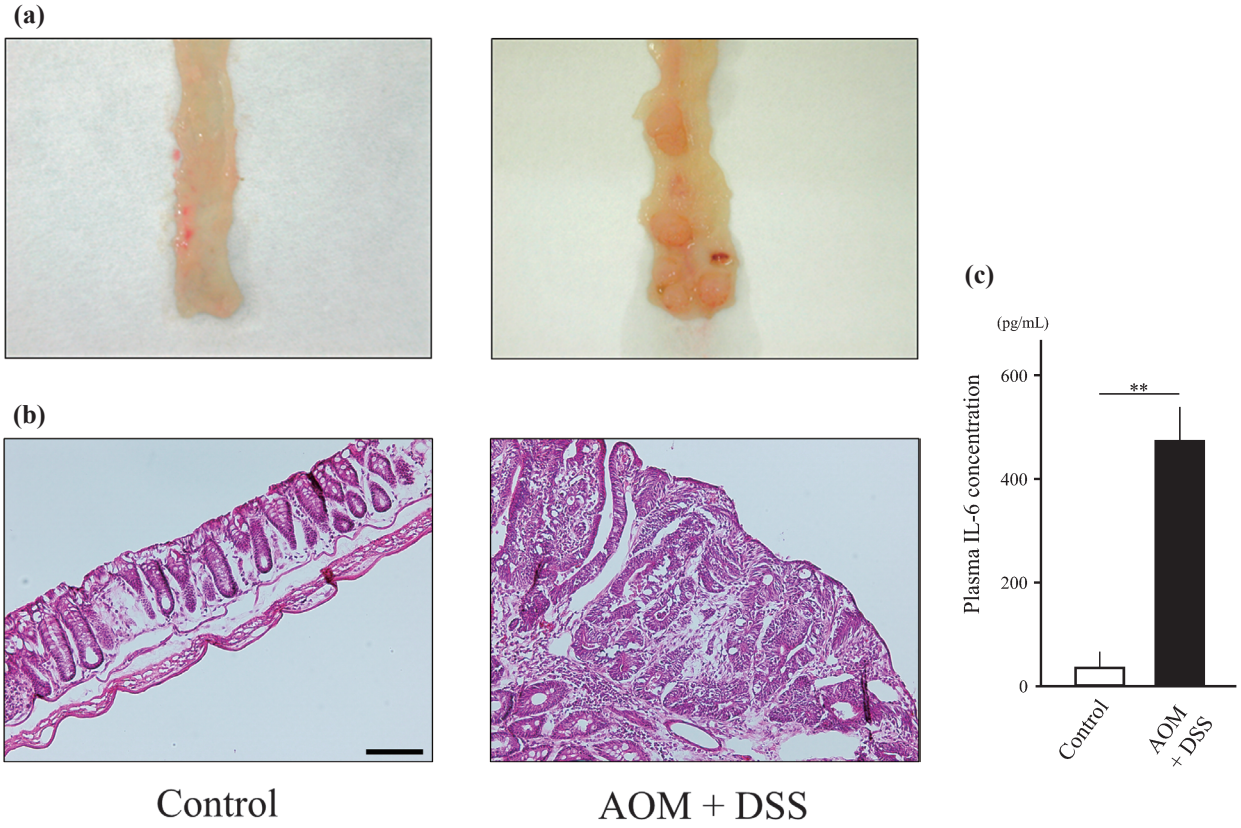

Fig. 1. (a) Macroscopic View and (b) Hematoxylin and Eosin Staining of Colon Tissue Sections after Treatment. (c) Plasma IL-6 Concentrations in the Induced Colon Carcinoma Models

Values represent the mean±standard deviation. The Student's *t*-test was used to compare the control group (n=5) to the AOM+DSS group (n=5). **p<0.01. Scale bar=100 μm.

(12.5 μg/lane) were loaded onto a 4–12% BIS-TRIS Bolt gel (Life Technologies, Carlsbad, CA, U.S.A.) and electrophoresed at 165 V for 30 min. After separation, the proteins were transferred onto a nitrocellulose membrane, using an iBlot Western blotting system (Life Technologies), which was subsequently blocked overnight with 5% skim milk at 4°C. After blocking, the membranes were then incubated at 25°C for 1 h with primary antibodies for type I collagen (1 : 1000; EMD Chemicals Inc., NJ, U.S.A.) or β-actin (1 : 5000; Sigma). The immune complexes on the membranes were then visualized using a horseradish peroxidase-conjugated secondary antibody (Dako Cytomation, Glostrup, Denmark) and ImmunoStar Zeta (Wako Pure Chemical Indistries, Ltd., Osaka, Japan). Images were acquired using Multi-Gauge software (FUJIFILM, SC, U.S.A.).

Statistical Analyses All data were presented as mean±standard deviation. Student's *t*-test was used to compare the means of the two groups, and differences were considered statistically significant at a *p*-value of <0.05.

RESULTS

Induction of Colon Carcinoma *via* AOM+DSS Treatment After 20 weeks of treatment, blood and tissue samples were obtained, and the colon carcinomas were observed macroscopically (Figs. 1a, b). Using the blood samples, we evaluated plasma IL-6 concentrations, as IL-6 is a critical tumor promoter during early colitis-associated carcinogenesis.[5] The results indicated that the plasma IL-6 concentrations in mice that were treated with AOM+DSS were significantly higher than those in the control mice (Fig. 1c); we conclude that these findings indicated carcinogenesis.

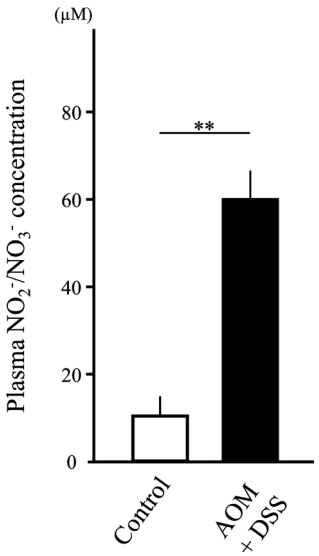

Fig. 2. Plasma Levels of NO_2^-/NO_3^- after Treatment

Values represent the mean±standard deviation. The Student's *t*-test was used to compare the control group (n=5) to the AOM+DSS group (n=5). **p<0.01.

Upregulation of Inducible Nitrous Oxide Synthase in AOM+DSS-Treated Mice After the treatment, plasma levels of NO_2^-/NO_3^- were measured for each mouse, as a surrogate marker for inducible nitric oxide synthase expression.

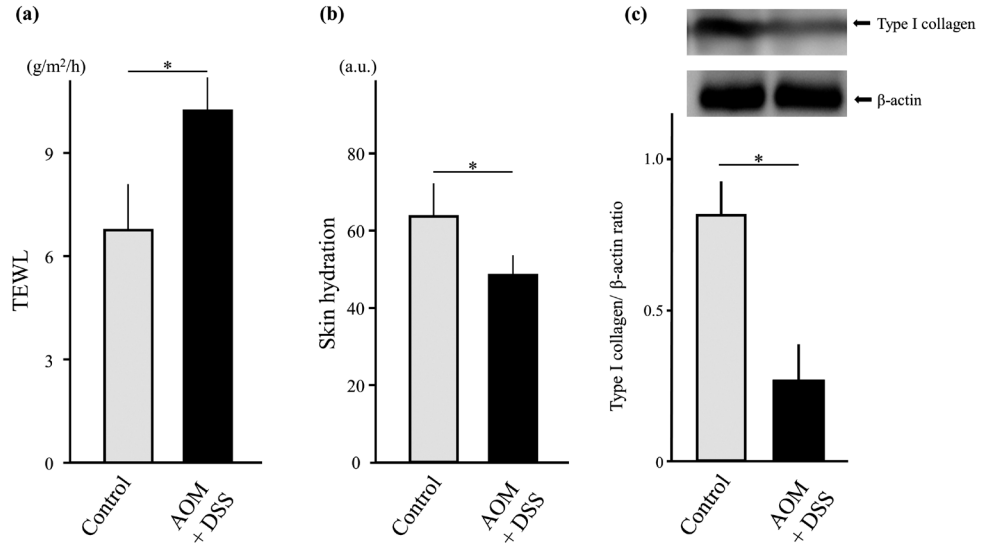

Fig. 3. Analysis of TEWL (a) and Skin Hydration (b) on the Dorsal Skin after Treatment; (c) Expression of Type I Collagen in the Skin

Values represent the mean±standard deviation. The Student's *t*-test was used to compare the control group (n=5) to the AOM+DSS group (n=5). *p<0.05. TEWL: transepidermal water loss.

In mice that were treated with AOM+DSS, the plasma NO_2^-/NO_3^- levels were significantly higher than those in the control mice (Fig. 2).

TEWL and Skin Hydration Significantly greater TEWL, and significantly lower skin hydration levels, were observed in mice that were treated with AOM+DSS, compared to those in the control mice (Figs. 3a, b).

Western Blot Analysis for Type I Collagen Mice that were treated with AOM+DSS had significantly lower expression of type I collagen, compared to that observed in the control mice (Fig. 3c).

DISCUSSION

Our results suggest that impaired skin barrier function develops in the presence of colon carcinoma, and that the onset of this disruption might be related to oxidative stress. In this context, oxidative stress is known to be related to skin damage *via* disruption of collagen I and other extracellular matrix components.[6,7] In a previous study, we have also reported that impaired skin barrier function was caused by mast cell degranulation in a mouse intestinal injury model.[2] In the present study, we confirmed that mast cells were present in the skin using toluidine blue staining, although no significant difference was observed between the mice that were treated with AOM+DSS and the control mice (data not shown). Therefore, it is necessary to clarify which causative molecular marker induces skin disruption in further studies.

Epidermal growth factor receptor inhibitors (EGFRI), such as cetuximab and panitumumab, have been used as treatments for colon carcinoma. However, one of the major adverse events that is related to EGFRI treatment is dermatologic toxicity, and this can place a burden on patients and medical staff.[8] In this context, EGFRI treatment can downregulate skin barrier function by regulating cytokines that modulate the EGFR signaling pathway in keratinocytes.[9,10] Thus, our results suggest that impaired skin barrier function has already occurred when colon carcinoma is present. Therefore, impaired skin barrier function that is caused by colon carcinoma might considerably accelerate any dermatological toxicity that is induced by EGFRI treatment.

In conclusion, our results provide evidence that support the morbid association of colon carcinoma with disruptions of normal skin barrier function. Furthermore, to our knowledge, this is the first investigation of the novel colon-to-skin barrier signal transduction pathway in mice with colon carcinoma. We hope to conduct further studies to clarify the relationship between keratinocytes and impaired skin barrier function when colon carcinoma is present.

Conflict of Interest The authors declare no conflict of interest.

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