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Original Article

Fecal microRNA223 as an indicator of recovery in chronic DSS colitis model in rats

Terutaka Kodama[1], Yuko Togashi[1], Naomi Matsutani[1], Seiichiro Kurashige[1], Toyohiko Aoki[2] and Yasuteru Otagiri[1]

[1]Toxicology and Pharmacokinetics Research Dept., Research Institute, EA Pharma Co., Ltd., 1-1 Suzuki-cho, Kawasaki-ku, Kawasaki-shi, Kanagawa 210-8681, Japan
[2]DIMS Institute of Medical Science, Inc., 64 Goura, Nishiazai, Azai-cho, Ichinomiya, Aichi, 491-0113, Japan

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ABSTRACT — Using a rat dextran sulfate sodium (DSS)-colitis model, we elucidated that the expression of miRNAs in colorectal tissues, plasma, and feces, particularly miR-31a-5p, miR-181b-5p, and miR-223-3p, could be used as noninvasive biomarkers to evaluate the reversibility of the model. We further investigated whether changes in miRNA levels were reproducible in chronic DSS-induced colitis in rats. Male SD rats were administered 5% DSS in drinking water for two cycles. Cycle 1 consisted of a 7-d dosing period and 14-d recovery period, followed by Cycle 2 consisting of a 5-d dosing period and 7-d recovery period. In-life parameters and the disease activity index (DAI) were respectively examined or calculated daily. Colon length and pathological changes were assessed postmortem in Cycle 2. A panel of nine miRNAs was also measured in colorectal tissues, plasma, and feces using digital PCR. The changes in DAI score and colon length were evident in Cycle 2. Erosive and inflammatory changes were observed in the colon and rectum following DSS treatment. At the end of the off-dose period of Cycle 2, the histological changes in the rectum worsened, while the colon changes showed recovery. The expression patterns of all miRNAs were almost the same in Cycle 2 when compared to those in a previous study (Kodama *et al*., 2021). Fecal miR-223-3p could be also a useful non-invasive indicator to evaluate the reversibility in chronic DSS-induced colitis in rats.

Key words: miRNA, digital PCR, Dextran sulfate sodium (DSS), Rats

INTRODUCTION

In recent years, several clinical and nonclinical studies on miRNA expression have been conducted. Patients with ulcerative colitis (UC) display significantly higher levels of miR-223 and miR-1246 than healthy volunteers (Verdier *et al*., 2020). Serum samples from patients with IBD showed higher levels of miR-16, miR-21, and miR-223 (Schönauen *et al*., 2018). The resveratrol-mediated attenuation of colitis may be regulated by miR-31 through the induction of Tregs, and miR-31 may serve as a therapeutic target for human colitis (Alrafas *et al*., 2020). In addition to clinical research, up- or downregulation of several miRNAs have been reported in patients with IBD. The administration of dextran sulfate sodium (DSS) to mice (Melgar *et al*., 2005) and rats (Kodama *et al*., 2021) resulted in toxicity in the large intestine, including inflammation and epithelial damage similar to IBD; thus, the DSS-induced colitis model is widely used as a nonclinical IBD model. Kodama *et al.* (2021) showed that in the rat DSS-induced colitis model, miR-31a, miR-181b, and miR-223 levels in plasma and feces were significant-

Correspondence: Terutaka Kodama (E-mail: terutaka_kodama@eapharma.co.jp)

ly reduced after DSS treatment, and those in feces were increased after the recovery period. The miRNA expression profiles dynamically changed either by overexpression (miR-181b, miR-31, miR-146b, miR-221, and miR-155) or by suppression (miR-34a) through NF-κB-STAT3 crosstalk in azoxymethane (AOM)/DSS-induced colon tumorigenesis in mice (El-Daly *et al*., 2019). Fecal miR-31a-5p, miR-181b-5p, and miR-223-3p could be used as non-invasive biomarkers translatable in animals and humans (Kodama *et al*., 2021). Multiple cycles of repeated DSS administration resulted in a chronic IBD model (Eichele and Kharbanda, 2017). In this study, we investigated changes in miRNA levels in a rat model of chronic IBD and examined sensitive indicators during the recovery of chronic DSS-induced colitis in rats.

MATERIALS AND METHODS

Animal study

Nineteen five-week-old male Sprague-Dawley rats (Crl:CD [SD]) were purchased from Charles River Japan Inc. (Shiga, Japan). The rats were individually housed in a room maintained at a controlled temperature (22 ± 3°C) and relative humidity (55 ± 15%), and a 12-hr light/dark cycle (07:00–19:00) in a barrier system. A standard commercial diet (MF; Oriental Yeast Co. Ltd., Tokyo, Japan) and tap water were provided *ad libitum*. After acclimatization for eight days, the rats were randomly allocated to four groups (n = 4 each in the control and DSS groups as the dosing groups and n = 4 each in the control and DSS groups as the recovery groups) based on their body weight. Animals that were not assigned to any study group were excluded.

DSS was obtained from MP Biomedicals (Santa Ana, CA, USA) and dissolved in tap water to obtain appropriate concentrations. Control rats received tap water only. DSS was administered to rats for two cycles. The dosing solution was administered in drinking water at daily doses of 0 (control) or 5% w/v DSS for 7 d. After the 7-d dosing, DSS administration was withdrawn for the next 14 d (Cycle 1). Doses of 0 (control) or 5 w/v% DSS were administered again daily for 5 d. After the 5-d dosing, DSS administration was withdrawn for the next 7 d (Cycle 2), and the dosing regimen is shown in Fig. 1. The DSS dosage levels were selected based on the results of a previous study (Kodama *et al*., 2021). During the dosing and recovery periods, the rats were observed once daily for general clinical signs and stools. All animals were weighed and food consumption and water intake were measured daily from the starting dose to the day of necropsy. The average DSS intake was 7758.27 mg/kg/d and 4712.42 mg/kg/d in Cycle 1 and Cycle 2 in the DSS group, respectively. The average values of water intake and body weight during the administration period of Cycle 1 were 33.3 g to 48.9 g and 248.4 g to 306.9 g, respectively, and those of Cycle 2 were 34.5 g to 43.4 g and 409.0 g to 418.9 g, respectively. The difference in the mean DSS intake between Cycle 1 and Cycle 2 was due to differences in body weight.

The disease activity index (DAI), defined as [the sum of scores of body weight gain, fecal condition, and bloody stool]/3, was calculated for each individual daily during the experimental period (Table 1).

Rats were sacrificed under isoflurane anesthesia and necropsied at the end of the administration or recovery period. The thoracic and intraperitoneal organs were examined macroscopically, and the large intestine (colon to anus) was excised. Colon length (the boundary between the cecum and the colon to the anus) was measured using a ruler. The large intestine was fixed in 10% buffered formalin. All tissues obtained were embedded in paraffin, sectioned, and stained with hematoxylin and eosin (HE). Histopathological examination of the large intestine was performed under a light microscope. Blood was collected from the abdominal aorta, and feces and tissues (distal colon to rectum) were obtained on each day of necropsy to measure the miRNAs. All experiments were conducted in conformity with the guide for animal experiments at the DIMS Institute of Medical Science, Inc., Aichi, Japan.

Measurement of miRNAs

Blood samples were obtained from the abdominal aorta of rats under isoflurane anesthesia on the day of necropsy. Blood was collected in tubes containing EDTA-2K, centrifuged at approximately 3,000 × *g* for 15 min at 4°C, and filtered. Plasma was collected and preserved below −70°C. Feces from the colon to the rectum were collected at necropsy and preserved below −70°C. Tissues were collected from the rectum and distal colon, which were cut into three 5-mm^2 pieces, left overnight at 2–10°C, immersed in RNA*later* (Thermo Fisher Scientific K.K., Tokyo, Japan), and preserved below −70°C.

Digital PCR was conducted on plasma, feces, and tissue samples. Briefly, total RNA was extracted with QIAzol using miRNeasy serum/plasma kit (QIAGEN K.K., Tokyo, Japan) for plasma, miRNeasy micro kit (QIAGEN K.K.) for tissues, and an RNeasy PowerMicrobiome kit (QIAGEN K.K.) for feces. cDNA for the miRCURY LNA miRNA PCR assay was synthesized using the miRCURY LNA RT kit (QIAGEN K.K., Catalog #339340). The synthesized cDNA was stored at −20°C. The cDNA was diluted 1:2000 depending on the miRNA and sample to

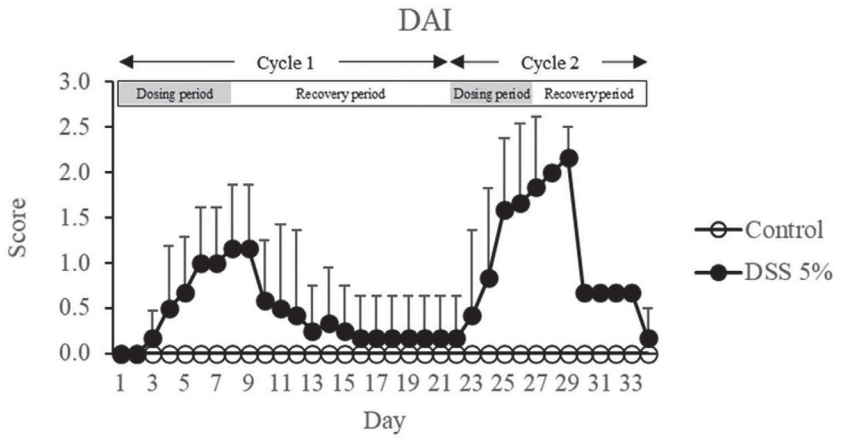

Fig. 1. DAI during the study (Mean + S.D.). N = 8/group during the first dosing period and recovery period in Cycle 1 and the second dosing period in Cycle 2. N = 4/group during the second recovery period in Cycle 2.

Table 1. Score for disease activity index.

Score	Body weight gain (%)	Fecal condition	Bloody stool
0	No decrease	Normal	Not detected
1	−5 to −1		
2	−10 to −5	Loose stool	
3	−20 to −10		
4	< −20	Diarrhea	Present

form a DNA template for PCR. After preparing the PCR solution with 2 × EvaGreen ddPCR SuperMix (Catalog #186-4033, Bio-Rad, Tokyo, Japan), a droplet was prepared using the QX200™ Droplet Digital™ PCR system (Catalog #1864001, Bio-Rad). PCR was conducted on a MiniAmp™ PCR System (Thermo Fisher Scientific K.K.) according to the manufacturer's instructions, and the fluorescence intensity was read using a QX200 Droplet Reader (Bio-Rad) and QuantaSoft software (Bio-Rad). The target genes evaluated were miR-16-3p, miR-21-5p, miR-31a-5p, miR-34a-5p, miR-146b-5p, miR-155-5p, miR-181b-5p, miR-221-5p and miR-223-3p. The fold-change of individual values relative to the mean value of the control group was calculated for each miRNA.

Statistical analysis

For the DAI score, colon length, and each miRNA level, mean values and standard deviation (S.D.) were calculated for each measurement day and for each group. In addition, for colon length and each miRNA in each group, the data were first analyzed using the F test (two-tailed, significance level: $p < 0.05$) to determine homogeneity of variance. When the variance was homogeneous, Student's t-test (two-tailed, significance level: $p < 0.05$) was used to compare the averages between the control and 5% DSS groups. When the variance was heterogeneous, Welch's test (two-tailed, significance level: $p < 0.05$) was used. As the minimum number of animals required for animal welfare was used in this study, a comprehensive evaluation was conducted by adding a biological evaluation to the statistical analysis.

RESULTS

Clinical observations

No deaths were noted in the control or 5% DSS groups during the experiment. Anal bleeding, as an abnormal clinical sign, was observed in six rats in the 5% DSS group during Cycles 1 and 2, and one animal became emaciated and anemic on days 26 and 27. Body weight and food intake tended to be lower in the 5% DSS group than in the control group, and loss of body weight was evident during the administration period of Cycle 2. The water intake tended to be higher in the 5% DSS group than in the control group (data not shown).

All animals in the 5% DSS group exhibited loose stool during the treatment period, and bloody stool was observed in four rats in Cycle 1. Although loose stool generally disappeared during the recovery period in Cycle 1, the same change was observed again in Cycle 2. Some animals had diarrhea in Cycle 1 or Cycle 2. Bloody stools were observed in six rats in Cycle 2. The DAI score increased in the 5% DSS group during DSS treatment then decreased, followed by a recovery period. Although the DAI score became 0.2 after completion of the recovery period in Cycle 1, the increased DAI score was reproduced by the second DSS treatment in Cycle 2. After the recovery peri-

Table 2. Colon length at the end of the administration and recovery periods in Cycle 2.

Test article	Control		DSS	
Dose (w/v%)	0		5	
Consecutive day of experiment	Day 27	Day 34	Day 27	Day 34
Day in Cycle 2	Day 6	Rec. Day 8	Day 6	Rec. Day 8
Number of animals	4	4	4	4
Colon length (cm)	21.6 ± 0.6	22.1 ± 0.8	17.4 ± 2.9ns	17.3 ± 1.1**

Data are shown as mean ± S.D. ** $p < 0.01$, ns: not significant ($p = 0.06$).

od in Cycle 2, the DAI score returned to 0.2; however, the speed of recovery was slow in Cycle 2 (Fig. 1).

Pathological findings in the colon and rectum

At necropsy in Cycle 2, no notable macroscopic changes were observed in DSS-treated rats at the end of the administration and recovery periods, except for one rat who manifested anemia as its first clinical sign, as evidenced by the pallor of the spleen, liver, and kidneys. The colon length tended to be shorter in the 5% DSS group at the end of the treatment in Cycle 2 than in the control group, and the shortened colon length was still evident in DSS-treated animals after the recovery period in Cycle 2 (Table 2). Histopathological examination revealed erosion and inflammatory cell infiltration in the rectum and colon of the 5% DSS group. One rat showed minimal erosion and inflammatory cell infiltration in the rectum, while the other three rats showed relatively higher erosion and inflammatory cell infiltration in the rectum (Table 3). At the end of the recovery period, the frequency and extent of erosion or inflammatory cell infiltration in the colon tended to be reversible. However, the changes in the rectum worsened even after the recovery period in Cycle 2.

miRNA levels in the colon, rectum, plasma, and feces

Figure 2 shows the miRNA levels in the colon, rectum, plasma, and feces of the control group in Cycle 2, which were obtained using digital PCR. Although there was a difference in absolute values depending on the sampling location, the levels on day 27 (day 6 in Cycle 2) and day 34 (recovery day 8) were similar, and no time-dependent difference was observed.

Figure 3 shows the miRNA levels in the colon, rectum, plasma, and feces. On day 6, colonic miRNA levels were significantly lower than those in each control group for all miRNAs except miR-155-5p. miR-155-5p showed similarly low values to those of the other miRNAs (Fig. 3A). Rectal miRNA levels were significantly lower for all miRNAs compared to those in each control group (Fig. 3B). Plasma miRNA levels were significantly lower than those in each control group for all miRNAs except for miR-16-3p and miR-221-5p. The miR-16-5p value was also similar to that of other miRNAs (Fig. 3C). Fecal miRNA levels were significantly lower for miR-31a-5p, miR-146b-5p, miR-181b-5p, and miR-221-5p compared to the levels in each control group. In addition, although the difference was not significant, miR-16-3p, miR-34a-5p, and miR-223-3p showed lower values, similar to those of the other miRNAs (Fig. 3D). On recovery day 8, colonic miRNA levels were significantly lower for miR-34a-5p and miR-221-5p compared to those in each control group (Fig. 3E). Rectal miRNA levels were significantly lower for miR-16-3p, miR-31a-5p, miR-34a-5p, miR-146b-5p, miR-181b-5p, miR-221-5p, and miR-223-3p than those in each control group. In addition, although the difference was not significant, miR-155-5p showed lower values, similar to those of these miRNAs (Fig. 3F). Plasma miRNA levels were not significantly different for all miRNAs compared to those in the control group (Fig. 3G). Fecal miRNA levels were significantly higher in the miR-31a-5p group than in the control group. In addition, although the difference was not significant, miR-16-3p and miR-223-3p showed values higher than or equal to those of miR-31a-5p (Fig. 3H). Most of the above changes in miRNA levels in the colon, rectum, plasma, and feces were generally reproducible when compared with a previous study (Kodama *et al*., 2021) in which the changes/deviations were relatively milder.

DISCUSSION

Treatment with DSS is known to induce colitis in rats and mice and is widely used as a nonclinical chemically induced IBD model (Melgar *et al*., 2005; Zhu *et al*., 2019). Previously, we investigated a reliable indicator of IBD using a rat DSS colitis model, and suggested that miRNAs from the colon, rectum, plasma, and feces are good biomarkers in a rat model (Kodama *et al*., 2021).

In this study, to confirm whether the changes in miRNA levels were a good indicator of recovery from chronic colitis, we investigated the parameter changes using a rat DSS-induced chronic colitis model; 5% DSS was admin-

Table 3. Histopathological findings in large intestine at the end of the administration and recovery periods in Cycle 2.

Test article		Control		DSS	
Dose (w/v%)		0		5	
Consecutive day of experiment		Day 27	Day 34	Day 27	Day 34
Day in Cycle 2		Day 6	Rec. Day 8	Day 6	Rec. Day 8
Number of animals		4	4	4	4
No remarkable changes		4	4	0	0
Colon, proximal					
Erosion, focal	Minimal	0	0	1	0
Infiltrate inflammatory cell, mucosal, focal	Minimal	0	0	1	0
Colon, mid					
Erosion, focal	Slight	0	0	2	0
Erosion, diffuse	Minimal	0	0	0	1
	Moderate	0	0	1	0
Infiltrate inflammatory cell, mucosal, focal	Slight	0	0	2	0
Infiltrate inflammatory cell, mucosal, diffuse	Minimal	0	0	0	1
	Slight	0	0	1	0
Colon, distal					
Erosion, diffuse	Minimal	0	0	0	2
	Slight	0	0	2	0
	Marked	0	0	1	0
Infiltrate inflammatory cell, mucosal, diffuse	Minimal	0	0	0	2
	Slight	0	0	2	0
Infiltrate inflammatory cell, submucosal, diffuse	Moderate	0	0	1	0
Rectum					
Erosion, focal	Minimal	0	0	1	0
Erosion, diffuse	Moderate	0	0	3	4
Infiltrate inflammatory cell, mucosal, focal	Minimal	0	0	1	0
Infiltrate inflammatory cell, mucosal, diffuse	Slight	0	0	2	0
	Moderate	0	0	1	1
Infiltrate inflammatory cell, submucosal, diffuse	Moderate	0	0	0	3

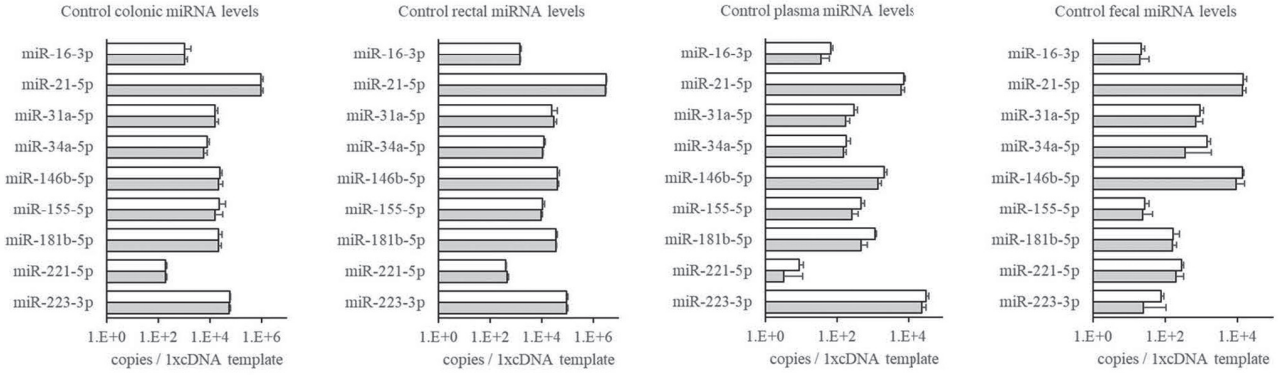

Fig. 2. miRNA levels in the colon, rectum, plasma, and feces in the control group in Cycle 2 (Mean + S.D.). The white bar represents the miRNA levels at the end of the dosing period (day 6). Gray bars represent miRNA levels at the end of the recovery period (recovery day 8). N = 4/group.

istered to rats with drinking water in two cycles of 7 or 5 d of treatment and 14 d of recovery between the cycles. During the experimental period, the general clinical signs, stool condition, body weight, food consumption, and water intake were monitored. Colon length, macro- and micro-pathological examination, and changes in miRNAs were evaluated after DSS treatment or after the recovery period in Cycle 2, and the results were then compared

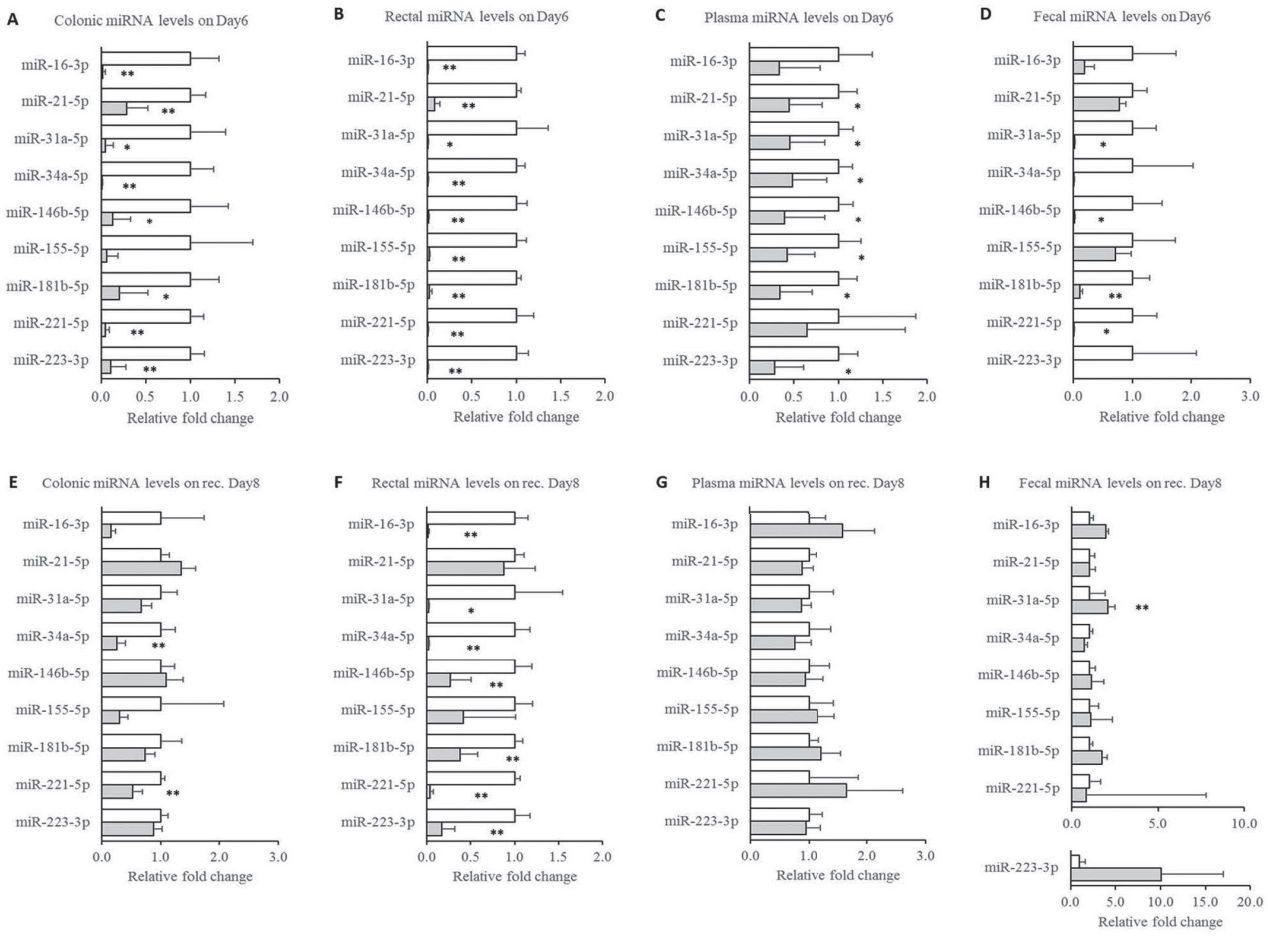

Fig. 3. miRNA levels in the colon, rectum, plasma, and feces in Cycle 2 (Mean + S.D.). White bars represent miRNA levels in the control group. Gray bars represent miRNA levels in the 5% DSS dosing group. N = 4/group. *: $p < 0.05$, **: $p < 0.01$.

with those of a previous study (Kodama *et al*., 2021). The comparative data between previous and current studies are summarized in Table 4.

Each cycle of 5% DSS administration resulted in a high DAI score. The DAI score was markedly higher in Cycle 2 than in Cycle 1; therefore, the effects of Cycle 1 may have persisted in Cycle 2. At the end of the administration period in Cycle 2, expression levels of miRNAs, except miR-221-5p in plasma and miR-21-5p and miR-155-5p in feces, were lower in the 5% DSS group than in the control group. Changes in plasma and fecal miRNAs are largely linked to colonic and rectal miRNA levels. However, the degree of reduction in plasma miRNA levels was lower than that in colon and rectal miRNA levels. The decrease in each miRNA after the end of treatment was almost consistent with the results of a previous report (Kodama *et al*., 2021) corresponding to the evaluation in Cycle 1. These selected plasma and fecal miRNAs, except for miR-21-5p, miR-155-5p, and miR-221-5p, were considered useful as safety biomarkers for DSS-induced colitis in rats.

At the end of the recovery period following DSS treatment, plasma miRNA had increased to approximately the same level as that in the control group, but this level was not especially high. For fecal miRNAs, an increase in miR-16-3p and miR-31a-5p was observed, but this increase was not profound. The histopathological changes in the colon tended to recover, but those of the rectum tended to worsen in the rectum at the end of a 1-week recovery period. In this study, the small increase in miRNA levels was thought to be due to insufficient recovery from gastrointestinal toxicity after the end of the recovery period of Cycle 2. However, despite the weak reversibility, only fecal miR-223-3p levels increased, which sug-

Table 4. Comparison of the effects of Cycle 1 and Cycle 2 after DSS administration.

Cycle		Cycle 1		Cycle 2	
Consecutive day of experiment		Day 8	Day 16	Day 27	Day 34
Day in each cycle		Day 8	Rec Day 8	Day 6	Rec Day 8
Maximum DAI score		1.2 (on Day9)	0.2 (on Day21)	2.2 (on Day 29)	0.2 (on Day34)
Colon length		−14%*	−14%*	−19%	−22%
Histopathology[a)]	Colon Proximal	-*	-*	Minimal	-
	Mid	Minimal*	-*	Slight	Minimal
	Distal	Minimal*	-*	Moderate	Minimal
	Rectum	Slight*	Slight*	Moderate	Moderate
miR-223-3p expression[b)]	Colon	0.029*	1.7*	0.11	0.89
	Rectum	0.014*	1.5*	4.1×10^{-3}	0.17
	Plasma	0.18*	1.3*	0.28	0.94
	Feces	0.36*	29*	0	10

-; no remarkable change

[a)]; as severity of colitis integrating inflammation and epithelial damage

[b)]; relative fold change to the concurrent controls

*data from Kodama *et al*. (2021)

gested that fecal miR-223-3p was a sensitive indicator.

miR-223 expression is upregulated during experimental IBD in mice. Overexpression of miR-223 attenuates experimental colitis, NOD-like receptor protein 3 (NLRP3) levels, and IL-1β release, thereby reducing IBD severity (Neudecker *et al*., 2017). Upregulating miR-223 by agomir administration alleviated colonic inflammation in a DSS-induced colitis mouse model, which was likely mediated by inhibiting the production of pro-inflammatory cytokines via the IL-6/STAT3 signaling pathway (Zhang *et al*., 2020). miR-223 regulates the differentiation and proliferation of granulocytes, macrophages, and dendritic cells by binding to specific targets. Additionally, miR-223 regulates pro-inflammatory or anti-inflammatory macrophage polarization. miR-223 can also bind to specific target genes to inhibit pro-inflammatory cytokines or inflammatory signals in these cells (Jiao *et al*., 2021). Based on this evidence, increased levels of miR-223-3p in the feces indicated suppression of inflammation in the large bowel during the recovery period. Fecal miR-223-3p is considered a sensitive biomarker that responds to the recovery tendency of rat gastrointestinal toxicity.

miR-223 is highly expressed in the serum and feces of patients with UC and Crohn's disease (CD), suggesting that miR-223 is involved in its regulation and could serve as a biomarker for UC and CD (Wang *et al*., 2016; Schönauen *et al*., 2018). miR-223 plays an important role in the development and progression of UC and CD. miR-223 expression is significantly increased in mucosal biopsy tissues of UC patients (Valmiki *et al*., 2017). In this study, fecal miR-223-3p levels decreased significantly after DSS administration and increased during the recovery period. On the contrary, the increase was presumed to indicate inflammation that patients should have recovered from previously. An elevated miR-223-3p level in IBD patients is considered to indicate immediate or current inflammation and ongoing recovery.

In this study, we investigated sensitive indicators of IBD using a chronic DSS-induced colitis model in rats and confirmed that the changes in selected miRNAs in acute DSS-induced colitis were reproducible. Thus, fecal miR-223-3p could be used as a non-invasive indicator to evaluate the reversibility of chronic DSS colitis in rats.

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Conflict of interest---- The authors declare that there is no conflict of interest.

REFERENCES

Alrafas, H.R., Busbee, P.B., Nagarkatti, M. and Nagarkatti, P.S. (2020): Resveratrol Downregulates miR-31 to Promote T Regulatory Cells during Prevention of TNBS-Induced Colitis. Mol. Nutr. Food Res., **64**, 1900633.

Eichele, D.D. and Kharbanda, K.K. (2017): Dextran sodium sulfate colitis murine model: an indispensable tool for advancing our understanding of inflammatory bowel diseases pathogenesis. World J. Gastroenterol., **23**, 6016-6029.

El-Daly, S.M., Omara, E.A., Hussein, J., Youness, E.R. and El-Khayat, Z. (2019): Differential expression of miRNAs regu-

lating NF-κB and STAT3 crosstalk during colitis-associated tumorigenesis. Mol. Cell. Probes, **47**, 101442.

Jiao, P., Wang, X.P., Luoreng, Z.M., Yang, J., Jia, L., Ma, Y. and Wei, D.W. (2021): miR-223: An Effective Regulator of Immune Cell Differentiation and Inflammation. Int. J. Biol. Sci., **17**, 2308-2322.

Kodama, T., Togashi, Y., Matsutani, N., Kurashige, S., Aoki, T. and Otagiri, Y. (2021): Expression of miRNAs in the colon, rectum, plasma, and feces in rats with dextran sulfate sodium. Fundam. Toxicol. Sci., **8**, 177-182.

Melgar, S., Karlsson, A. and Michaelsson, E. (2005): Acute colitis induced by dextran sulfate sodium progresses to chronicity in C57BL/6 but not in BALB/c mice: correlation between symptoms and inflammation. Am. J. Physiol. Gastrointest. Liver Physiol., **288**, G1328-G1338.

Neudecker, V., Haneklaus, M., Jensen, O., Khailova, L., Masterson, J.C., Tye, H., Biette, K., Jedlicka, P., Brodsky, K.S., Gerich, M.E., Mack, M., Robertson, A.A., Cooper, M.A., Furuta, G.T., Dinarello, C.A., O'Neill, L.A., Eltzschig, H.K., Masters, S.L. and McNamee, E.N. (2017): Myeloid-derived miR-223 regulates intestinal inflammation via repression of the NLRP3 inflammasome. J. Exp. Med., **214**, 1737-1752.

Schönauen, K., Le, N., von Arnim, U., Schulz, C., Malfertheiner, P. and Link, A. (2018): Circulating and Fecal microRNAs as Biomarkers for Inflammatory Bowel Diseases. Inflamm. Bowel Dis., **24**, 1547-1557.

Valmiki, S., Ahuja, V. and Paul, J. (2017): MicroRNA exhibit altered expression in the inflamed colonic mucosa of ulcerative colitis patients. World J. Gastroenterol., **23**, 5324-5332.

Verdier, J., Breunig, I.R., Ohse, M.C., Roubrocks, S., Kleinfeld, S., Roy, S., Streetz, K., Trautwein, C., Roderburg, C. and Sellge, G. (2020): Faecal Micro-RNAs in Inflammatory Bowel Diseases. J. Crohn's Colitis, 110-117.

Wang, H., Zhang, S., Yu, Q., Yang, G., Guo, J., Li, M., Zeng, Z., He, Y., Chen, B. and Chen, M. (2016): Circulating MicroRNA223 is a New Biomarker for Inflammatory Bowel Disease. Medicine (Baltimore), **95**, e2703.

Zhang, J., Wang, C., Zhen, G.Z., Da, B., Zhu, W. and Li, Q. (2020): miR-223 improves intestinal inflammation through inhibiting the IL-6/STAT3 signaling pathway in dextran sodium sulfate-induced experimental colitis. Immun. Inflamm. Dis., **9**, 319-327.

Zhu, L., Gu, P. and Shen, H. (2019): Protective effects of berberine hydrochloride on DSS-induced ulcerative colitis in rats. Int. Immunopharmacol., **68**, 242-251.