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Mucosal concentrations of *N*-acetyl-5-aminosalicylic acid related to endoscopic activity in ulcerative colitis patients with mesalamine

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Abstract

Background and Aim: 5-Aminosalicylic acid (5-ASA) is a fundamental treatment for mild-to-moderate ulcerative colitis (UC). 5-ASA is taken up into the colonic mucosa and metabolized to *N*-acetyl-5-ASA (Ac-5-ASA). Few studies have assessed whether mucosal 5-ASA and Ac-5-ASA concentrations are associated with endoscopic remission. This study aimed to investigate differences in 5-ASA and Ac-5-ASA concentrations according to endoscopic activity.
Methods: This single-center, prospective, cross-sectional study was conducted between March 2018 and February 2019. UC patients who were administered with 5-ASA medication for at least 8 weeks before sigmoidoscopy were enrolled. Mucosal 5-ASA and Ac-5-ASA concentrations were measured using liquid chromatography with tandem mass spectrometry. The primary endpoint was defined as the difference in mucosal concentrations of 5-ASA and Ac-5-ASA, according to the Mayo endoscopic subscore (MES).
Results: Mucosal concentrations were analyzed in 50 patients. In the sigmoid colon, the median 5-ASA concentration in patients with MES of 0 (17.3 ng/mg) was significantly higher than MES $\geq$ 1 (6.4 ng/mg) (P = 0.019). The median 5-ASA concentrations in patients with Ulcerative Colitis Endoscopic Index of Severity $\leq$ 1 (16.4 ng/mg) were also significantly higher than in patients with Ulcerative Colitis Endoscopic Index of Severity $\geq$ 2 (4.63 ng/mg) (P = 0.047). In the sigmoid colon, the concentration of Ac-5-ASA was higher in patients with MES of 0 (21.2 ng/mg) than in patients with MES $\geq$ 1 (5.81 ng/mg) (P = 0.022).
Conclusions: The present study showed that mucosal Ac-5-ASA concentrations, as well as 5-ASA concentrations, are higher in UC patients with endoscopic remission. Ac-5-ASA may be useful for a biomarker of 5-ASA efficacy.

Introduction

Ulcerative colitis (UC) is a chronic and idiopathic inflammatory bowel disease (IBD). 5-Aminosalicylic acid (5-ASA) is useful for both induction and maintenance of remission in UC patients. Although the specific mechanism of action of 5-ASA is unclear, 5-ASA is known to be absorbed poorly from the colon, and it exerts its beneficial effect by acting topically on the colonic mucosa. 5-ASA is inactivated to *N*-acetyl-5-ASA (Ac-5-ASA) in the colonic mucosa and is considered to be excreted in the urine and feces.[1] These facts suggest that a high concentration of 5-ASA is associated with clinical efficacy, while Ac-5-ASA is an unnecessary substance in the colonic mucosa.

Recently, many studies have been conducted on the relationship between drug concentrations and clinical efficacy in IBD. For instance, several studies have reported that trough concentration is

correlated with clinical symptoms, endoscopic activity, histological activity, and biomarkers such as fecal calprotectin (FC) and C-reactive protein in IBD patients receiving anti-tumor necrosis factor-α antibodies.[2–4] Like anti-tumor necrosis factor-α antibody, 5-ASA concentrations in colonic mucosa are related to clinical symptoms and endoscopic evaluations. Frieri *et al*. reported that the colonic concentration of 5-ASA was negatively associated with an endoscopic activity, histological severity, and soluble interleukin-2 receptor levels.[5] Our group also previously reported a negative correlation between mucosal 5-ASA concentrations and the disease activity index in patients with UC, suggesting that clinical efficacy may depend on the colonic mucosal concentration of 5-ASA.[6]

Mucosal healing has emerged as part of a treat-to-target strategy in clinical practice for UC patients,[7,8] and patients who achieve endoscopic remission have improved outcomes.[9–11] In the recent statement from the International Organization of Inflammatory Bowel Disease,[12] complete endoscopic remission, such as Mayo endoscopic subscore (MES) of 0, is considered the optimal target, indicating that a higher level of therapeutic effect is required.

Although endoscopic severity has been associated with a mucosal concentration in patients receiving 5-ASA, few studies have assessed the relationship between the mucosal concentration of 5-ASA and its metabolite, Ac-5-ASA, and complete endoscopic remission, such as MES of 0. In particular, concerning Ac-5-ASA, the pharmacological effects are considered to be limited. Therefore, the relationship between therapeutic effects and Ac-5-ASA is unknown. This study aimed to investigate the difference in 5-ASA and Ac-5-ASA concentrations according to the level of endoscopic activity.

Methods

Study design. This single-center, prospective, cross-sectional study was conducted between March 2018 and February 2019 at the clinic of the Division of Gastroenterology and Hepatology, Department of Internal Medicine, Keio University Hospital (Tokyo, Japan). All patients underwent colonoscopy without pretreatment. On the morning of the sigmoidoscopy examination, oral administration of 5-ASA was given as usual. In this study, types of 5-ASA included salazosulfapyridine (Salazopyrin), time-dependent 5-ASA (Pentasa), pH-dependent 5-ASA (Asacol), and multimatrix system 5-ASA (Lialda), which were permitted for use in Japan in 2018.

The Protocol Committee defined the criteria for the termination of this study. The study could be terminated if it was decided that the continuation of the study or recruitment of participants was too difficult or if the Ethics Committees recommended it. The study could also be terminated if the aims of the study were achieved before the planned number of investigated cases was reached or the aims were achieved before the end of the scheduled period. The committee also defined the criteria for discontinuation of individual participants when they wished to cease participation in this study or when the eligibility criteria were not met after enrollment.

Patients. Eligible patients were at least 20 years of age with a diagnosis of UC, according to the diagnostic criteria defined by the Research Group of Inflammatory Bowel Disease at the Ministry of Health, Labour and Welfare in Japan, and mild-to-moderate disease activity. Patients with UC who were treated with 5-ASA medications for at least 8 weeks before sigmoidoscopy were eligible for enrollment. We requested patients who were scheduled to undergo colonoscopy to participate in this study. Exclusion criteria were as follows: (i) administration of 5-ASA topical therapy within a week, to eliminate the effect of topical therapy on mucosal 5-ASA concentration; (ii) steroid preparations (suppositories, enemas, oral steroid preparations, and steroid injectable preparations) or biologics within 2 weeks of endoscopic enrollment; (iii) poor medication adherence (less than 75%); (iv) a history of colon surgery; and (v) serious renal disorder/liver disorder.

Endoscopic procedure. All participants underwent sigmoidoscopy without pretreatment to measure mucosal 5-ASA and Ac-5-ASA concentrations. Endoscopic severity was assessed using conventional white light imaging (Olympus Medical Systems Co., Tokyo, Japan). Endoscopic disease activity was assessed by two trained endoscopists and was scored according to the Mayo endoscopic score and the Ulcerative Colitis Endoscopic Index of Severity (UCEIS). Biopsies were performed to extract approximately 10 mg of tissue from the sigmoid colon and rectum (two or three biopsies were performed from each segment) for the measurement of 5-ASA concentrations. Biopsy specimens were obtained from the most severe inflammation site in each segment. Additionally, the mucous membrane at the biopsy site was washed before biopsy so that it does not contain 5-ASA on the mucosal epithelium in the sample.

Measurement of the mucosal concentration of 5-aminosalicylic acid and N-acetyl-5-aminosalicylic acid. The measurement of the mucosal concentration of 5-ASA and Ac-5-ASA is included in Appendix S1.

Clinical assessments. Demographic data and disease characteristics of the patients were collected at the enrollment of this study. Clinical disease activity was scored according to the Mayo score[13] and Lichtiger index[14] on the day of the sigmoidoscopy procedure. Blood tests were conducted, including the measurement of C-reactive protein levels. All clinical information, including MES and UCEIS, were entered into the electric data capture system by the investigators (SRL Medisearch Inc., Tokyo, Japan). The information regarding FC levels and concentration of 5-ASA was blinded to the investigators when this study was finalized. The stool samples were collected within 2 days before the colonoscopic procedure. FC (Calprotectin Mochida, Mochida Pharmaceutical, Tokyo, Japan) was measured using enzyme-linked immune sorbent assay kits.[15] All study data were sent to the statistician (Biostatistics Unit at Clinical and Translational Research Center, Keio University School of Medicine, Tokyo, Japan).

Sample size. The sample size for this study was determined, taking account of its feasibility. We defined the number of registered patients as 60. The number of patients with UC who visit Keio University Hospital annually is about 1800, and the number of patients who undergo colonoscopy is about 700 every year, so it

was considered possible to register 60 patients. *Post hoc power* analyses indicated that if Cohen's effect size was high (e.g. 0.80), 50 patients (25 patients per arm) represented 80% power with two-tailed 5% alpha in a comparison between two groups defined with drug concentration.

Endpoints. The primary endpoint was defined as the difference in mucosal concentration of 5-ASA and Ac-5-ASA between patients with endoscopic remission (MES of 0) and patients without endoscopic remission (MES $\geq$ 1). Additionally, the difference in mucosal concentrations of 5-ASA and Ac-5-ASA was also compared between patients with UCEIS $\leq$ 1 and patients with UCEIS $\geq$ 2. MES and UCEIS were evaluated in the sigmoid colon and rectum and compared with the concentrations of 5-ASA and Ac-5-ASA at the mucosal collection site. The secondary endpoint was to compare the correlation between concentrations of 5-ASA and Ac-5-ASA. As an exploratory analysis, the mucosal concentrations of 5-ASA and Ac-5-ASA were compared among the patients receiving time-dependent 5-ASA, pH-dependent 5-ASA, and multimatrix system 5-ASA.

Statistical analysis. All data were collected up to February 2019 and finalized in February 2019. Categorical variables were described as absolute numbers and relative frequencies using percentages. Continuous variables were described as medians and interquartile ranges (IQRs). The median mucosal 5-ASA and Ac-5-ASA levels were compared using a Mann–Whitney analysis or Kruskal–Wallis analysis. Correlation between mucosal 5-ASA concentrations and endoscopic severity (MES, UCEIS) or FC levels was analyzed using the Spearman's rank correlation coefficient. The significance level for each test was 5% (two tailed). All analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA).

Ethical considerations. This study was approved by the Ethics Committee of Keio University School of Medicine (no. 20170324) and was conducted in accordance with the principles of the Declaration of Helsinki. All participants gave written informed consent.

Results

Patient profiles. Fifty-seven UC patients who had been treated with 5-ASA medications for at least 8 weeks before the study were enrolled, and 56 patients participated between March and December 2018. To clarify the differences in mucosal 5-ASA concentrations among patients who use various oral 5-ASA medications, two patients receiving salazosulfapyridine were excluded, and another four patients receiving low-dose 5-ASA were also excluded from the full analysis set (Fig. 1). Furthermore, one patient was excluded because of no efficacy data. The demographic factors at baseline for the full analysis set population are shown in Table 1. Although most patients maintained clinical remission, 21 patients (42%) did not achieve endoscopic remission. The median duration of 5-ASA administration was 7.5 years.

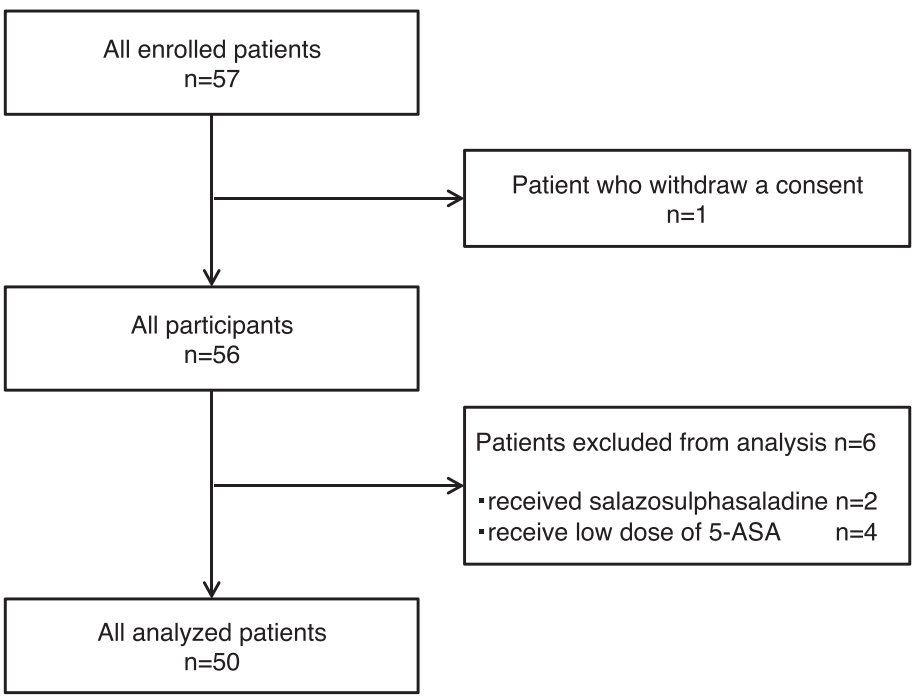

Fig 1 Flowchart of the study methodology. A total of 57 patients with ulcerative colitis participated in this study. Among them, one patient revoked consent before undergoing colonoscopy. The remaining 56 patients were included. Because our study protocol aimed for a population of 50 patients, we excluded patients using salazosulfapyridine and patients using low-dose 5-aminosalicylic acid (5-ASA).

Table 1 Baseline characteristics of the 50 patients in this study

	n = 50
Male, *n* (%)	24 (48.0)
Age (years), median (IQR)	44.0 (33.0–55.0)
Duration of disease (years), median (IQR)	7.5 (5.0–12.0)
Extent of disease	
Extensive, *n* (%)	23 (46.0)
Left-sided, *n* (%)	15 (30.0)
Rectal, *n* (%)	12 (24.0)
Duration of 5-ASA administration (years), median (IQR)	7.5 (4.0–12.0)
Total Mayo score, median (IQR)	0.0 (0.0–3.0)
Partial Mayo score, median (IQR)	0.0 (0.0–1.0)
Mayo endoscopic score, *n* (%)	
0	29 (58.0)
1	5 (10.0)
2	15 (30.0)
3	1 (2.0)
Lichtiger index, median (IQR)	2.0 (1.0–3.0)
5-ASA, *n* (%)	
Time-dependent 5-ASA	16 (32.0)
pH-dependent 5-ASA	18 (36.0)
Multimatrix system 5-ASA	16 (32.0)
Fecal calprotectin, median (IQR)	111.0 (45.5–632.0)

5-ASA, 5-aminosalicylic acid; IQR, interquartile range.

The mucosal concentration of 5-aminosalicylic acid in the sigmoid colon and rectum. In the sigmoid colon, the median 5-ASA concentration was 17.3 ng/mg (IQR: 4.30–71.2) and 1.95 ng/mg (IQR: 0.14–11.7) in patients with MES of 0 and ≥ 1, respectively (Fig. 2). The median 5-ASA concentrations in the sigmoid colon in patients with MES of 0 were significantly higher than that in patients with MES ≥ 1 ($P = 0.019$). Similar results were obtained when the relationship between 5-ASA concentrations and endoscopic severity (using UCEIS) was investigated. The median 5-ASA concentrations in patients with UCEIS ≤ 1 (16.4 ng/mg [IQR: 4.04–68.9]) were also significantly higher than that in patients with UCEIS ≥ 2 (4.63 ng/mg [IQR: 0.14–11.9]) ($P = 0.047$) (Fig. 3).

In the rectum, the median 5-ASA concentrations in patients with MES of 0 (36.9 ng/mg [IQR: 0.51–73.7]) were also higher than that in patients with MES ≥ 1 (13.5 ng/mg [IQR: 5.41–38.3]); however, there were no significant differences between the groups ($P = 0.45$) (Fig. 2).

The mucosal concentration of N-acetyl-5-aminosalicylic acid in the sigmoid colon and rectum. In the sigmoid colon, the median Ac-5-ASA concentration in patients with MES of 0 (21.2 ng/mg [IQR: 13.1–38.7]) was significantly higher than that in patients with MES ≥ 1 (5.81 ng/mg [IQR: 2.22–29.1]) ($P = 0.022$) (Fig. 2). It was also significantly

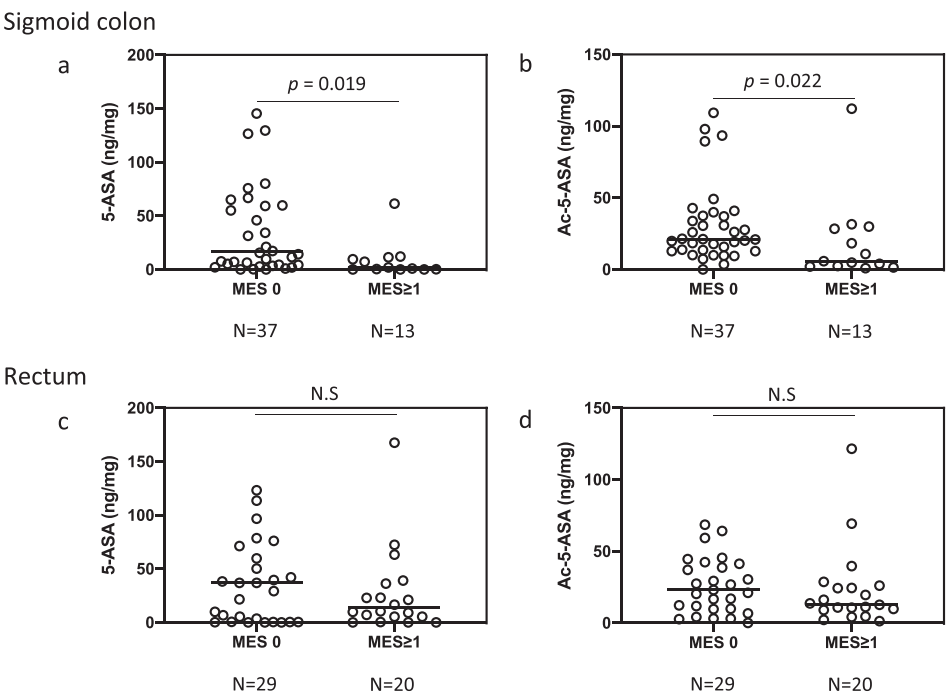

Fig 2 (a) Mucosal concentrations of 5-aminosalicylic acid (5-ASA) and Mayo endoscopic activity (MES) in the sigmoid colon. Five data points are outside the axis limits. (b) Mucosal concentrations of *N*-acetyl-5-ASA (Ac-5-ASA) and MES in the sigmoid colon. One data point is outside the axis limits. (c) Mucosal concentrations of 5-ASA and MES in the rectum. Three data points are outside the axis limits. (d) Mucosal concentrations of Ac-5-ASA and MES in the rectum. One data point is outside the axis limits.

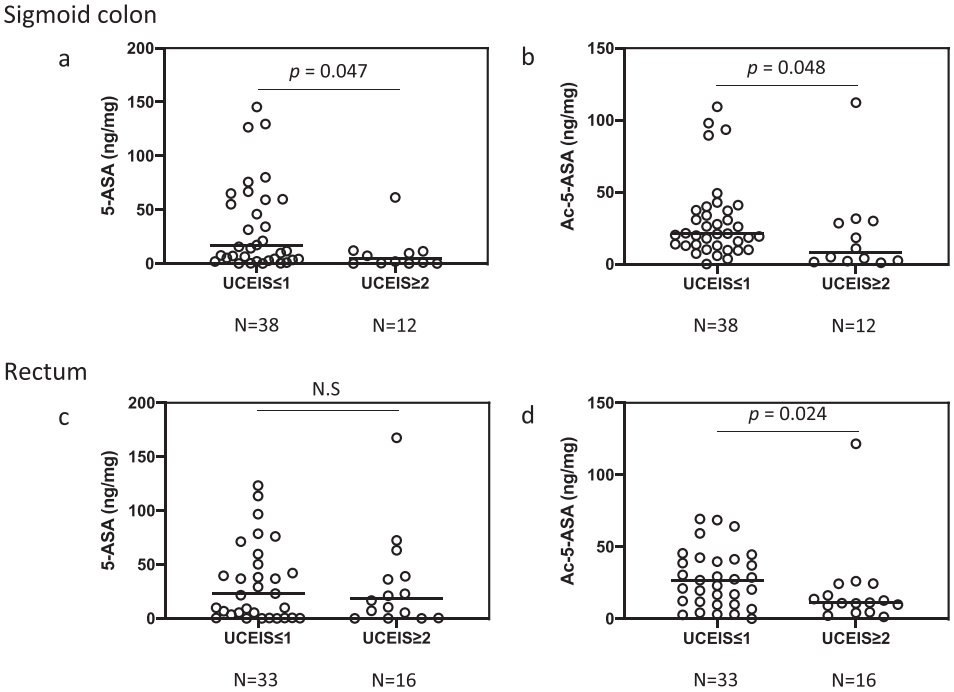

Fig 3 (a) Mucosal concentrations of 5-aminosalicylic acid (5-ASA) and Ulcerative Colitis Endoscopic Index of Severity (UCEIS) in the sigmoid colon. Five data points are outside the axis limits. (b) Mucosal concentrations of *N*-acetyl-5-ASA (Ac-5-ASA) and UCEIS in the sigmoid colon. One data point is outside the axis limits. (c) Mucosal concentrations of 5-ASA and UCEIS in the rectum. Three data points are outside the axis limits. (d) Mucosal concentrations of Ac-5-ASA and UCEIS in the rectum. One data point is outside the axis limits.

higher in patients with a UCEIS $\leq$ 1 (21.1 ng/mg [IQR: 12.8–38.1]) than in patients with a UCEIS $\geq$ 2 (7.81 ng/mg [IQR: 2.14–29.5]) (P = 0.048) (Fig. 3). Although the rectal Ac-5-ASA concentration in patients with MES of 0 was comparable with patients with MES $\geq$ 1, Ac-5-ASA concentration in the rectum was significantly higher in patients with a UCEIS $\leq$ 1 than in patients with a UCEIS $\geq$ 2 (P = 0.024) (Fig. 3).

Figure 4 shows the correlation between 5-ASA and Ac-5-ASA concentrations in the sigmoid colon and rectum. 5-ASA concentrations were significantly associated with Ac-5-ASA concentration in the sigmoid colon (r = 0.762, $P < 0.001$) and rectum (r = 0.714, $P < 0.001$).

In the sigmoid colon, Spearman's correlation coefficients for 5-ASA concentration and MES and 5-ASA concentration and UCEIS were −0.321 (P = 0.024) and −0.287 (P = 0.044), respectively. Furthermore, the coefficients for Ac-5-ASA concentration and MES and Ac-5-ASA concentration and UCEIS were −0.380 (P = 0.007) and −0.305 (P = 0.031), respectively. On the other hand, there was no association between 5-ASA concentration and MES or 5-ASA concentration and UCEIS in the rectum.

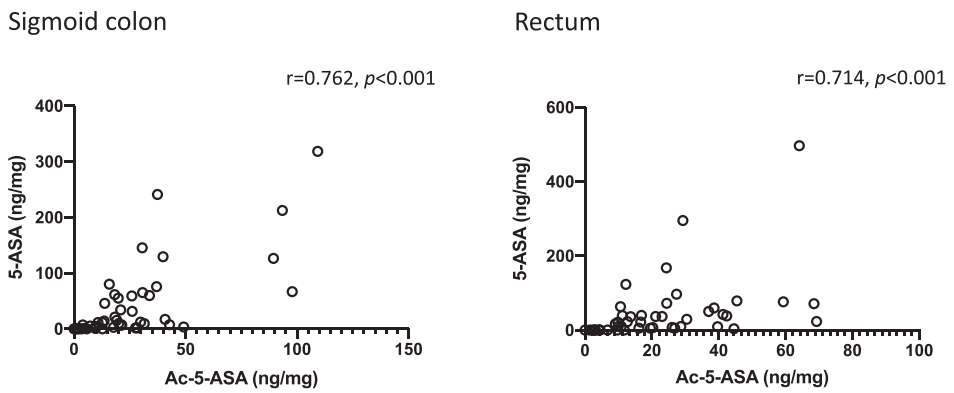

Fig 4 5-aminosalicylic acid (5-ASA) concentration correlates with *N*-acetyl-5-ASA (Ac-5-ASA) concentration in the sigmoid colon (r = 0.762, $P < 0.001$) and the rectum (r = 0.714, $P < 0.001$).

Relationship between mucosal 5-aminosalicylic acid concentration and fecal calprotectin level. Next, we assessed whether FC level was associated with mucosal 5-ASA concentrations. The median 5-ASA concentration in the sigmoid colon tended to be higher in patients whose FCP level was less than 240 μg/g (cutoff value predicting MES ≤ 1 in the manufacturer's instructions) compared with patients whose FCP level was more than the cutoff value (17.3 vs. 5.2, $P = 0.147$). However, the FCP level was not associated with 5-ASA concentration in the rectum.

Mucosal 5-aminosalicylic acid and N-acetyl-5-aminosalicylic acid concentration in patients receiving various 5-aminosalicylic acid medications. Table 2 shows the 5-ASA concentrations among patients receiving different 5-ASA formulations. As an exposure analysis, the differences in mucosal 5-ASA and Ac-5-ASA concentration in patients with different oral 5-ASA were assessed. All patients were taking the maximum dose of 5-ASA permitted in healthcare practice. 5-ASA and Ac-5-ASA concentrations in both the sigmoid colon and the rectum were comparable among patients who received different 5-ASA formulations. However, 5-ASA concentrations in both the sigmoid colon and the rectum tended to be lower in patients receiving time-dependent 5-ASA than patients receiving pH-dependent 5-ASA or multimatrix system 5-ASA. Ac-5-ASA concentrations among patients receiving different 5-ASA formulations were comparable in the sigmoid colon and rectum.

Discussion

To our knowledge, this is the first study to demonstrate that patients with complete endoscopic remission in terms of MES of 0 or UCEIS ≤ 1 have higher mucosal concentrations of 5-ASA and Ac-5-ASA than those with mild-to-moderate endoscopic severity. Recent studies have shown that patients with MES of 1 experience recurrence more frequently than those with MES of 0,[16] and further additional treatment improves clinical outcomes in patients with MES of 1, indicating that MES of 0 and 1 should be considered as distinct clinical outcomes.[17] Therefore, MES of 0 and 1 should be clearly distinguished, although a previous study that evaluated the relationship between 5-ASA mucosal concentration and endoscopic activity defined MES of 0 and 1 as inactive periods.[5] In our study, we strictly defined complete endoscopic remission as only MES of 0 and investigated the difference in 5-ASA mucosal concentrations in the colonic mucosa between patients with MES of 0 and ≥ 1. Our prospective study revealed that patients with MES of 0 had higher 5-ASA concentration than those with MES of 1.

In this study, we did not confirm that a high concentration of 5-ASA was associated with endoscopic severity in the rectum, although the median 5-ASA concentrations in patients with MES of 0 tended to be higher than that in patients with MES ≥ 1. Because the samples were collected from the most severe point of inflammation in the rectum, the site of the biopsy performed in the rectum varied among patients. Anatomical characteristics of the biopsy site such as Rs, Ra, and Rb probably influenced the amount of 5-ASA taken up into the colonic mucosa. Consequently, in the rectum, mucosal concentrations of 5-ASA and Ac-5-ASA varied between biopsy sites and showed no statistical difference. Anatomical differences may explain why median rectal 5-ASA and Ac-5-ASA mucosal concentrations were higher than those in the sigmoid colon.

For the first time in this study, we examined the relationship between Ac-5-ASA mucosal concentration and endoscopic activity. Although studies have investigated the effects of colonic mucosal concentration of 5-ASA on clinical and biological efficacy, the effects of Ac-5-ASA on disease activity have not received much attention. 5-ASA is metabolized by *N*-acetyltransferase that catalyzes the transfer of an acetyl moiety from acetyl-coenzyme A to the nitrogen of the 5-ASA to form the metabolite Ac-5-ASA in the mucosa.[18] Although a previous double-blind controlled study showed a therapeutic effect of Ac-5-ASA, the effects of Ac-5-ASA on clinical efficacy are minimal, compared with the effects of 5-ASA in the colonic mucosa.[19] Interestingly, the present study

Table 2 The mucosal concentration of 5-ASA and Ac-5-ASA among patients receiving different 5-ASA formulations

Concentration of 5-ASA	Drug	*N*	Median (IQR)	*P* value
Sigmoid colon				
5-ASA conc.	Time-dependent 5-ASA	16	5.3 (0.6–25.8)	0.36
	pH-dependent 5-ASA	18	11.5 (5.2–65.0)	
	Multimatrix system 5-ASA	16	18.3 (3.2–68.6)	
Ac-5-ASA conc.	Time-dependent 5-ASA	16	24.5 (9.3–35.7)	0.94
	pH-dependent 5-ASA	18	18.8 (10.8–31.6)	
	Multimatrix system 5-ASA	16	18.8 (9.5–35.5)	
Rectum				
5-ASA conc.	Time-dependent 5-ASA	15	5.4 (0.4–72.5)	0.61
	pH-dependent 5-ASA	18	21.3 (6.8–38.3)	
	Multimatrix system 5-ASA	16	36.6 (3.8–67.3)	
Ac-5-ASA conc.	Time-dependent 5-ASA	15	24.4 (9.7–45.4)	0.45
	pH-dependent 5-ASA	18	14.4 (9.9–27.4)	
	Multimatrix system 5-ASA	16	22.7 (8.7–37.8)	

5-ASA, 5-aminosalicylic acid; Ac-5-ASA, *N*-acetyl-5-aminosalicylic acid; conc., concentration; IQR, interquartile range.

also showed that mucosal concentrations of Ac-5-ASA are higher in patients with complete endoscopic remission. This finding was obtained despite the previous assumption that Ac-5-ASA, a metabolite of 5-ASA, has few pharmacological effects on the colonic mucosa. As described earlier, we speculated that Ac-5-ASA concentration was not associated with endoscopic severity when we planned the present study. Contrary to our expectations, our study showed that high mucosal concentration of Ac-5-ASA was more frequently observed in patients with MES of 0 and patients with UCEIS of 0–1 compared with patients with mild-to-moderate endoscopic severity. Furthermore, we also confirmed that the concentration of 5-ASA and Ac-5-ASA was positively associated with the sigmoid colon and rectum. High concentrations of both 5-ASA and Ac-5-ASA were associated with MES of 0, and this can be explained by the fact that Ac-5-ASA remains as a metabolite of 5-ASA after contributing to the suppression of inflammation in patients with endoscopic remission. It is possible that the presence of high doses of 5-ASA in the local mucosa exerts a therapeutic effect in patients in remission. However, 5-ASA may be easily taken up and metabolized in sites without inflammation. Therefore, the concentration of its metabolite, Ac-5-ASA, is also higher in patients without mucosal inflammation. It is difficult to determine whether high concentrations of 5-ASA and Ac-5-ASA in the mucosa are the cause or the result of endoscopic remission.

In this study, we compared three different 5-ASA formulations administered in the maximum dose permitted in Japan. To directly compare the 5-ASA concentrations in patients receiving time-dependent 5-ASA, pH-dependent 5-ASA, and multimatrix system 5-ASA, patients who used 5-ASA topical therapy were excluded from this study. 5-ASA concentrations in both the sigmoid colon and the rectum were comparable among patients who received different 5-ASA formulations. However, 5-ASA concentrations tended to be lower in patients receiving time-dependent 5-ASA than patients receiving pH-dependent 5-ASA or multimatrix system 5-ASA, and this was consistent with the results of previous studies.[20,21] In Japan, the manufacturer's maximum dose of time-dependent, pH-dependent, or multimatrix system 5-ASA is 4.0, 3.6, and 4.8 g, respectively. We compared the concentrations in patients receiving the maximum dose of the three different formulations to answer the questions concerning real clinical practice; however, similar trends were observed when we eliminated the effects of differences in 5-ASA dose by comparing the median concentrations of 5-ASA per gram (data not shown). In the future, it should be clarified whether differences in 5-ASA and Ac-5-ASA mucosal concentrations for the various 5-ASA formulations are associated with differences in clinical efficacy.

There were some limitations to our study. First, the number of participants was relatively small; however, our sample size was satisfactory to assess the correlation between endoscopic severity and mucosal concentration. Second, the association between the clinical severity and 5-ASA concentrations was not assessed because most of the enrolled patients maintained clinical remission.

In conclusion, this is the first study to demonstrate that mucosa without inflammation had higher concentrations of 5-ASA and Ac-5-ASA compared with that in mucosa with mild-to-moderate inflammation. Although Ac-5-ASA, a metabolite of 5-ASA, is thought to have few pharmacological effects in the colonic mucosa, the present study showed that mucosal concentrations of Ac-5-ASA are higher in patients with endoscopic remission.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1. Measurement of mucosal concentration of 5-ASA and Ac-5-ASA