Regular Article

Influence of Pharmaceutical Formulation on the Mucosal Concentration of 5-Aminosalicylic Acid and *N*-Acetylmесalamine in Japanese Patients with Ulcerative Colitis

Yuki Yamamoto,^{a,b} Satoshi Masuda,^{*a,b,c} Hiroshi Nakase,^{d,e} Minoru Matsuura,^e Shihoko Maruyama,^a Tadakazu Hisamatsu,^{f,g} Yasuo Suzuki,^g and Kazuo Matsubara^a

^aDepartment of Clinical Pharmacology and Therapeutics, Kyoto University Hospital; 54 Kawaharacho, Shogoin, Sakyo-ku, Kyoto 606–8507, Japan; ^bDepartment of Clinical Pharmacology and Biopharmaceutics, Graduate School of Pharmaceutical Sciences, Kyushu University; 3–1–1 Maidashi, Higashi-ku, Fukuoka 812–8582, Japan; ^cDepartment of Pharmacy, Kyushu University Hospital; 3–1–1 Maidashi, Higashi-ku, Fukuoka 812–8582, Japan; ^dDepartment of Gastroenterology and Hepatology, Sapporo Medical University School of Medicine; Minami 1-jo Nishi 17-chome, Chuo-ku, Sapporo 060–8556, Japan; ^eDepartment of Gastroenterology and Hepatology, Graduate School of Medicine, Kyoto University; 54 Kawaharacho, Shogoin, Sakyo-ku, Kyoto 606–8507, Japan; ^fThe Third Department of Internal Medicine, Kyorin University School of Medicine; 6–20–2 Shinkawa, Mitaka 181–8611, Tokyo, Japan; and ^gDepartment of Internal Medicine, Toho University Sakura Medical Center; 564–1 Shimoshizu, Sakura, Chiba 285–8741, Japan.

Received July 24, 2018; accepted October 10, 2018; advance publication released online October 24, 2018

The efficacy of 5-aminosalicylic acid (5-ASA) as the first-line therapy for ulcerative colitis (UC) is determined by the extent of drug delivery to the inflamed region. Moreover, differences among the various formulations influence delivery of the drug. In this study, we examined the clinical significance of colonic mucosal concentrations of 5-ASA and *N*-acetylmесalamine (Ac-5-ASA) in UC patients receiving a pH-dependent or time-dependent release formulation of 5-ASA. The subjects were 67 patients with UC who were treated with a pH-dependent or time-dependent formulation of 5-ASA between December 2011 and April 2014. A retrospective observational analysis of clinical outcomes was performed using the clinical activity index (CAI) obtained on the day of biopsy. Colonic mucosal concentrations of 5-ASA and Ac-5-ASA in biopsy samples were measured by LC-tandem mass spectrometry/mass spectrometry. Patients who were treated with the pH-dependent formulation had higher colon mucosal concentrations of 5-ASA than those who were treated with the time-dependent formulation. Additionally, 5-ASA concentration was significantly higher in patients with CAI scores ≤ 3 . A higher concentration of Ac-5-ASA was achieved with the time-dependent formulation than with the pH-dependent formulation. Furthermore, patients with CAI scores ≤ 3 had higher concentrations of 5-ASA than those with CAI scores ≥ 4 . The colonic mucosal concentration of 5-ASA in patients with UC is influenced by the pharmaceutical formulation and the remission status of UC.

Key words pH-dependent formulation; time-dependent formulation; clinical activity index; *N*-acetylmесalamine

INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory disease with symptoms such as ulcers in the large intestinal mucosa, diarrhea, and mucous and blood in stool associated with erosion. UC is also characterized by repeated remission and redevelopment. Treatment of UC was initially limited to surgical resection of the affected part of the intestinal canal, which was a physical and mental burden on patients. Recent use of 5-aminosalicylic acid (5-ASA or mesalazine), steroids, immunosuppressants, calcineurin inhibitors, and biologicals allows for the management of UC with drug therapy alone. However, there has been a recent increase in the number of UC patients, and cases that are non-responsive to drug therapy have been identified. These patients still require resection of the intestinal canal, which has a significant negative effect on QOL.

5-ASA is the standard drug for the treatment of UC.⁽¹⁾ It has strong anti-inflammatory effects that depend on its concentration in mucous membranes. As a result, it is important to achieve a high 5-ASA concentration in mucous membranes to maintain remission of UC.^(2–5) When administered as an oral time-dependent release formulation, half of it is absorbed in

the small intestine. This reduces the efficacy of 5-ASA for treating UC since the major lesions are found in the large intestine, unlike Crohn's disease, which mainly affects the small intestine. In contrast, the efficacy of a pH-dependent release formulation of 5-ASA is dependent on pH changes in the digestive tract. 5-ASA is released mainly at pH 7 or higher from the ileum to the large intestine when it is coated with Eudragit®-S (Evonik Industries, Essen, Germany).⁽⁶⁾ This coating improves the anti-inflammatory effect of 5-ASA, as the concentration of the drug increases at UC lesion sites.

The effects of different formulations of 5-ASA on the colon mucosa are clear⁽⁷⁾; however, their efficacies in Japanese patients have not been examined. Because the inactive metabolite *N*-acetylmесalamine (Ac-5-ASA) is mainly excreted into the urine,⁽⁸⁾ the sum of the mucosal 5-ASA and Ac-5-ASA levels may reflect the total exposure of 5-ASA after oral administration. Therefore, in this study, we compared the effects of time-dependent and pH-dependent formulations of 5-ASA and Ac-5-ASA on the colon mucosa in Japanese patients with UC. This was done by performing biopsies of tissues from the large intestine and analyzing clinical activity index (CAI), which is a disease activity index for UC.⁽⁹⁾

*To whom correspondence should be addressed. e-mail: satomsdb@pharm.med.kyushu-u.ac.jp

PATIENTS AND METHODS

Materials 5-ASA and 4-aminosalicylic acid (4-ASA) were purchased from Wako Pure Chemical Industries, Ltd., Inc. (Osaka, Japan). 4-ASA was used as an internal standard. Ac-5-ASA was purchased from Toronto Research Chemicals (Toronto, Canada). The concentrations of 4-ASA, 5-ASA, and Ac-5-ASA (Fig. 1) were measured by LC-tandem mass spectrometry/mass spectrometry (LC-MS/MS). The LC-MS/MS system comprised a HPLC machine (LC-10AS; Shimadzu Corp., Kyoto, Japan), a column (Cosmosil®, 5C18-MS-II, 2.0×150 mm; Nacalai Tesque, Kyoto, Japan), and an MS/MS detector (API4000; Applied Biosystems, Foster City, CA, U.S.A.).

Patients The subjects enrolled in the study were 67 patients with UC who were treated with time-dependent (Pentasa® tablets; KYORIN Pharmaceutical Co., Ltd., Tokyo, Japan) (1.5–4.0 g/d) or pH-dependent (Asacol® tablets; ZERIA Pharmaceutical Co., Ltd., Tokyo, Japan) (2.4–4.8 g/d) formulations of 5-ASA from December 2011 to April 2014 at Kyoto University Hospital (Kyoto, Japan). Written informed consent was received when we undergo periodic sigmoidoscopy from all patients for participation in the study, and the patients treated with topical formulation were excluded. Each patient underwent periodic sigmoidoscopy, in which mucosal biopsy samples (about 1-mm cube each) were collected from three areas in the rectum. The samples were put in a microtube, immediately frozen in liquid nitrogen, and stored at –80°C until analysis to determine the concentrations of 5-ASA and its primary metabolite *N*-acetylmесalamine (Ac-5-ASA). This study was conducted in accordance with the Declaration of

Helsinki and its amendments, and was approved by the Ethics Committee of Kyoto University Graduate School and Faculty of Medicine.

Measurement of the Concentrations of 5-ASA and Ac-5-ASA Each frozen mucosal biopsy specimen was weighed and transferred into a separate protein LoBind® tube (Eppendorf Co., Tokyo, Japan). Next, 275 μL of 50% acetonitrile containing 0.1% formic acid and 25 μL of 400 ng/mL 4-ASA solution were added to the contents of the tube. The mixture was then homogenized ultrasonically and centrifuged at 14000 rpm for 5 min at room temperature with a bench-top Eppendorf® centrifuge machine. Afterwards, 150 μL of the supernatant was filtered through a 4-mm polytetrafluoroethylene filter (COSMONICE® filter [Organic Solvents], pore size of 0.45 μm, Nacalai Tesque) and the concentrations of 5-ASA and Ac-5-ASA in the filtrate were determined. HPLC was performed using 0.1% formic acid and acetonitrile containing 0.1% formic acid (30:70) as the mobile phase at a flow rate of 0.2 mL/min. The injection volume was set at 10 μL. Selected reaction monitoring transitions in the positive ion mode were *m/z* 154.20→*m/z* 80.10 for 4-ASA, *m/z* 154.20→*m/z* 108.10 for 5-ASA, and *m/z* 196.20→*m/z* 136.10 for Ac-5-ASA. Calibration curves constructed using peak areas were linear over a range of 0.0–50 ng/mL for 5-ASA and Ac-5-ASA.¹⁰⁾

Statistical Analysis Statistical analysis was performed on the mean of the values obtained from analyzing the three samples from each patient. Prior to the analysis, each measured concentration (ng/g tissue) was divided by the dose of 5-ASA (g/d) that was administered the previous day. 5-ASA concentrations in the active phase of UC tend to be lower than those in the remission phase.⁷⁾ Therefore, the concentrations of

Fig. 1. Chemical Structures of 4-ASA, 5-ASA and Ac-5-ASA

Table 1. Patient Characteristics

	CAI ≤3		<i>p</i> Value	CAI ≥4		<i>p</i> Value
	pH-Dependent	Time-dependent		pH-Dependent	Time-dependent	
Number	33	12		14	8	
Age (years)						
Median	39	37	0.97	43	42.5	0.68
Range	16–79	18–65		21–71	32–64	
Sex (male/female)	23/10	10/2	0.47	7/7	4/4	1.00
UC extent						
Rectal	9	1		4	1	
Life-sided	4	2		4	4	
Extended colon	19	9		6	3	
Unknown	1					
Mayo endoscopic subscores						
Mean	0.48	0.25	0.24	2.07	2.50	0.16
S.D.	0.62	0.45		0.73	0.53	

p Value was calculated by unpaired *t*-test.

Table 2. Dosage of 5-ASA (g/d)

	CAI ≤3				CAI ≥4			
	pH-Dependent		Time-dependent		pH-Dependent		Time-dependent	
	Dose (g/d)	N	Dose (g/d)	N	Dose (g/d)	N	Dose (g/d)	N
			1.50	1			1.50	1
	2.40	21	2.25	6	2.40	1	2.25	
	3.60	10	3.00	2	3.60	13	3.00	1
	4.80	2	4.00	3	4.80		4.00	6
p Value	0.541				0.857			

p Value was calculated by unpaired *t*-test.

Fig. 2. Influence of CAI and Formulation on the Concentration/Dose (C/D) Ratio of 5-ASA (A) and Ac-5-ASA (B) in UC Patients with CAI ≤3
The bar shows the median for the 5-ASA or Ac-5-ASA C/D ratio in each group.

5-ASA in patients with CAI scores ≤3, which is the standard level for a clinical response,¹¹⁾ were compared to those in patients with CAI scores ≥4. This was based on the examination of CAI values and Mayo endoscopic subscores¹²⁾ in electronic health records at the time of the biopsies. Statistical analyses were performed using GraphPad Prism® (version 5; GraphPad Software, Inc., La Jolla, CA, U.S.A.). A non-parametric test was performed for data that did not show a normal distribution.

RESULTS

No statistically significant differences were observed in age, sex, UC severity, or dose of 5-ASA between the two groups, or between patients whose CAI scores were ≤3 and those whose CAI scores were ≥4. In addition, the average Mayo endoscopic subscores in the two groups were similar (Tables 1, 2).

For patients with CAI scores ≤3 who were treated with the pH-dependent and time-dependent formulations, the mucosal concentration/dose (C/D) ratios (median (range)) of 5-ASA were 0.21 (0.04–11.2) and 0.08 (0.03–1.52) ng/mL, respectively. The difference between the values was found to be statistically significant (*p* = 0.019 by the Mann–Whitney *U* test) (Fig. 2A). However, the mucosal C/D ratios of Ac-5-ASA in the same group of patients were 0.60 (0.02–9.31) and 0.13 (<0.01 (not detected)–3.43) ng/mL, respectively (Fig. 2B). Furthermore, the mucosal 5-ASA/Ac-5-ASA concentration ratios were 1.53 (0.05–18.50) and 0.47 (not detected–20.71) for the pH-dependent and time-dependent formulations, respectively. The

Fig. 3. Influence of CAI and Formulation on the Concentration/Dose (C/D) Ratio of 5-ASA/Ac-5-ASA in UC Patients with CAI ≤3
The bar shows the median for the 5-ASA/Ac-5-ASA C/D ratio.

difference between the values was found to be statistically significant (*p* = 0.040 by the Mann–Whitney *U* test) (Fig. 3).
For patients with CAI scores ≥4 who were treated with the pH-dependent and time-dependent formulations, the mucosal C/D ratios of 5-ASA were 0.18 (0.02–3.27) and 0.12 (not detected–2.56) ng/mL, respectively (Fig. 4A), whereas the mucosal C/D ratios of Ac-5-ASA were 0.40 (0.06–15.9) and

Fig. 4. Influence of CAI and Formulation on the Concentration/Dose (C/D) Ratio of 5-ASA (A) and Ac-5-ASA (B) in UC Patients with CAI ≥ 4

The bar shows the median for the 5-ASA or Ac-5-ASA C/D ratio in each group.

Fig. 5. Influence of CAI and Formulation on the Concentration/Dose (C/D) Ratio of 5-ASA/Ac-5-ASA in UC Patients with CAI ≥ 4

The bar shows the median for the 5-ASA/Ac-5-ASA C/D ratio.

1.01 (0.03–6.54) ng/mL, respectively (Fig. 4B). Additionally, the mucosal 5-ASA/Ac-5-ASA concentration ratios were 0.81 (0.11–2.67) and 0.19 (0.02–2.45) for the pH-dependent and time-dependent formulations, respectively. The difference between the values was statistically significant ($p = 0.024$) according to the Mann–Whitney U test (Fig. 5).

DISCUSSION

5-ASA is the therapeutically active moiety of sulfasalazine and the recommended standard drug for the treatment of inflammatory bowel disease, which includes UC and Crohn's disease. Basically, 5-ASA is rapidly and well absorbed when administered as an instillation in the proximal part of the small intestine. Therefore, development of a 5-ASA formulation is usually aimed at maximizing the pharmacological effect of the drug by ensuring that it reaches the disease site, which could be any part of the gut in Crohn's disease or the colorectal site in UC.^{8,13} In the past two decades, several formulations have been developed for improving the absorption of 5-ASA. These include Pentasa®, which is a unique formulation in which the active ingredient is contained in coated microgranules. This formulation allows for a prolonged release of 5-ASA throughout the intestinal tract, from the duodenum to the rectum, following oral administration. On the other

hands, Asacol® is a pH-dependent formulation of 5-ASA. It is a delayed release enteric-coated tablet that releases the active ingredient in the colon. Based on these characteristics, we referred to Pentasa® and Asacol® as being “time-dependent” and “pH-dependent,” respectively, in the present study.

We examined the effects of the 5-ASA formulations and disease status based on CAI values obtained following oral administration of the formulations to the patients, as well as the delivery of the administered drug to the colonic mucosa. The constitution of the patient groups in the present study was considered appropriate because no significant difference in Mayo endoscopic subscores was found between the two groups. The mucosal C/D ratio of 5-ASA was higher in patients who received the pH-dependent release formulation than it was in patients who were treated with the time-dependent release formulation. In addition, the difference was noticeable in patients with CAI scores ≤ 3 compared to patients with CAI scores ≥ 4 . These results are consistent with previous findings, which suggested that the pH-dependent release of 5-ASA results in a higher concentration of 5-ASA in the large intestine.^{6,7} In a previous clinical study in which maintenance of remission and induction of remission in patients with UC were investigated, no statistically significant differences were found in the effects of a pH-dependent 5-ASA formulation (2.4 g/d) and a time-dependent 5-ASA formulation (2.25 g/d) with respect to efficacy and safety. However, among patients with proctitis-type UC, decreases in UC disease activity index were significantly different when the effects of the pH-dependent formulation (2.4 g/d) were compared to those of a placebo, but not when the effects of the time-dependent formulation (2.25 g/d) were compared to those of a placebo. The findings showed that the pH-dependent release of 5-ASA results in a higher concentration of 5-ASA in the large intestine than time-dependent release does. The results of the present study corroborate those of previous studies.¹⁴

The mucosal C/D ratio of 5-ASA/Ac-5-ASA was higher in those who were treated with the pH-dependent formulation than it was in those who received the time-dependent formulation. This was caused by more 5-ASA being delivered to the large intestine from the pH-dependent formulation than from the time-dependent formulation. Therefore, the pH-dependent formulation may be more effective in delivering 5-ASA to disease sites in patients with a higher level of inflammation.

In the present study, the large interindividual variation in

the mucosal C/D ratio of 5-ASA and Ac-5-ASA were observed. The biopsy specimens were collected through the daily clinical setting during endoscopic examination, and therefore, some physicochemical factors might influence it such as the time-lag between drug intake and endoscopic examination, and the peristaltic movement and the status of collected mucosa and so on. Furthermore, the mucosal concentrations of 5-ASA can differ substantially among individuals regardless of the formulation, which might be due to the activity of *N*-acetyltransferase (NAT). NAT metabolizes 5-ASA into its inactive metabolite Ac-5-ASA in the intestinal mucosa and liver. Ac-5-ASA is excreted mainly in urine, whereas the remnant is excreted in feces.^{8,15)} NAT1 and NAT2 are two NAT isozymes in humans. NAT1 is thought to be involved in the metabolism of 5-ASA.^{16,17)} Genetic polymorphism may cause a decrease in NAT1 activity.^{18,19)} It is also known that there are racial differences in *NAT1* gene polymorphism. In the Japanese population, four NAT1 alleles have been mentioned, which occur in a different frequency among Caucasians.²⁰⁾ Therefore, the relationship between *NAT1* gene polymorphism and the therapeutic effect of 5-ASA can be investigated in a future study.

In addition, the pH of intestinal luminal fluid may be lower in the active phase of UC than in the remission phase,²¹⁾ which can affect pH-dependent drug release. This is considered to be the reason why there was no difference in the C/D ratio of 5-ASA in patients with CAI scores ≥ 4 (Fig. 4A) despite a significant difference in the C/D ratio of 5-ASA between the two formulations in patients with CAI scores ≤ 3 (Fig. 2A).

This may explain the tendency for a lower C/D ratio of 5-ASA in the colonic mucosa in patients with CAI scores ≥ 4 compared to those with CAI scores ≤ 3 after treatment with a pH-dependent formulation. Therefore, if the effect of a pH-dependent formulation is insufficient, it may be necessary to change to a time-dependent formulation or use a combination of an enema and a suppository. The number of patients with CAI scores ≥ 4 was small in the present study. Therefore, differences in 5-ASA concentration between patients with CAI scores ≤ 3 and those with CAI scores ≥ 4 might be clearer when a larger number of patients with high UC activity are studied.

The present study is the first to compare two different 5-ASA formulations in humans with respect to the resulting mucosal concentrations of 5-ASA and Ac-5-ASA. In addition, it is the first to compare the two formulations in patients with CAI scores ≤ 3 and those with CAI scores ≥ 4 . This study has shown that, the pH-dependent formulation can be used to achieve higher concentrations of 5-ASA in the colonic mucosa. However, regardless of the type of formulation, 5-ASA concentrations might be affected by NAT activity and the level of inflammation. Therefore, dose adjustment and changing formulations may be necessary in the treatment of UC to meet individual patient needs. Further studies using a larger population of patients with various CAI and Mayo endoscopic subscores are required to confirm the results obtained.

Acknowledgments This work was supported in part by a Grant-in-Aid for Scientific Research (KAKENHI) (15H04666 to S. M., and 24928017 to Y. Y.) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), and by a Health and Labour Sciences Research Grant for research on intractable diseases from the Ministry of Health, Labour

and Welfare of Japan (Investigation and Research for Intractable Inflammatory Bowel Disease) (H. N., T. H., and Y. S.).

Conflict of Interest Tadakazu Hisamatsu received a research Grant from Mitsubishi Tanabe Pharma Corporation, EA Pharma Co., Ltd., AbbVie GK, JIMRO Co., Ltd., Pfizer Inc., Mochida Pharmaceutical Co., Ltd. Hiroshi Nakase received a research Grant from Hoya group Pentax Medical, Boehringer Ingelheim GmbH, Bristol Myers Squibb Company.

REFERENCES

- 1) Frieri G, Pimpo MT, Palumbo GC, Onori L, Viscido A, Latella G, Galletti B, Pantaleoni GC, Caprilli R. Rectal and colonic mesalazine concentration in ulcerative colitis: oral vs. oral plus topical treatment. *Aliment. Pharmacol. Ther.*, **13**, 1413–1417 (1999).
- 2) De Vos M, Verdievel H, Schoonjans R, Praet M, Bogaert M, Barbier F. Concentrations of 5-ASA and Ac-5-ASA in human ileocolonic biopsy homogenates after oral 5-ASA preparations. *Gut*, **33**, 1338–1342 (1992).
- 3) Fockens P, Mulder CJ, Tytgat GN, Blok P, Ferwerda J, Meuwissen SG, Tuynman HA, Dekker W, Gasthuis K, van Hees PA. Dutch Pentasa Study Group. Comparison of the efficacy and safety of 1.5 compared with 3.0 g oral slow-release mesalazine (Pentasa) in the maintenance treatment of ulcerative colitis. Dutch Pentasa Study Group. *Eur. J. Gastroenterol. Hepatol.*, **7**, 1025–1030 (1995).
- 4) Frieri G, Giacomelli R, Pimpo M, Palumbo G, Passacantando A, Pantaleoni G, Caprilli R. Mucosal 5-aminosalicylic acid concentration inversely correlates with severity of colonic inflammation in patients with ulcerative colitis. *Gut*, **47**, 410–414 (2000).
- 5) Naganuma M, Iwao Y, Ogata H, Inoue N, Funakoshi S, Yamamoto S, Nakamura Y, Ishii H, Hibi T. Measurement of colonic mucosal concentrations of 5-aminosalicylic acid is useful for estimating its therapeutic efficacy in distal ulcerative colitis: comparison of orally administered mesalamine and sulfasalazine. *Inflamm. Bowel Dis.*, **7**, 221–225 (2001).
- 6) Hirayama M, Toda R, Ozaki T, Hasegawa J, Nakamura T, Naraki Y, Haraguchi Y, Hori Y, Tanaka T, Takei M, Mera Y, Yoshii K, Kawabata Y. Concentration dependence of 5-aminosalicylic acid pharmacological actions in intestinal mucosa after oral administration of a pH-dependent formulation. *Mol. Pharm.*, **8**, 1083–1089 (2011).
- 7) D'Incà R, Paccagnella M, Cardin R, Pathak S, Baldo V, Giron MC, Sturniolo GC. 5-ASA colonic mucosal concentrations resulting from different pharmaceutical formulations in ulcerative colitis. *World J. Gastroenterol.*, **19**, 5665–5670 (2013).
- 8) Nielsen HO, Bondesen S. Kinetics of 5-aminosalicylic acid after jejunal instillation in man. *Br. J. Clin. Pharmacol.*, **16**, 738–740 (1983).
- 9) Lichtiger S, Present DH, Kornbluth A, Gelernt I, Bauer J, Galler G, Michelassi F, Hanauer S. Cyclosporine in severe ulcerative colitis refractory to steroid therapy. *N. Engl. J. Med.*, **330**, 1841–1845 (1994).
- 10) Nobilis M, Vybiralova Z, Sladkova K, Lisa M, Holcapek M, Kvetina J. High-performance liquid-chromatographic determination of 5-aminosalicylic acid and its metabolites in blood plasma. *J. Chromatogr. A*, **1119**, 299–308 (2006).
- 11) Yokoyama Y, Watanabe K, Ito H, Nishishita M, Sawada K, Okuyama Y, Okazaki K, Fujii H, Nakase H, Masuda T, Fukunaga K, Andoh A, Nakamura S. Factors associated with treatment outcome, and long-term prognosis of patients with ulcerative colitis undergoing selective depletion of myeloid lineage leucocytes: a prospective multicenter study. *Cytotherapy*, **17**, 680–688 (2015).
- 12) Schroeder KW, Tremaine WJ, Ilstrup DM. Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. A randomized study. *N. Engl. J. Med.*, **317**, 1625–1629 (1987).

- 13) Brunner M, Assandri R, Kletter K, Tschurlovits M, Corrado ME, Villa R, Eichler HG, Muller M. Gastrointestinal transit and 5-ASA release from a new mesalazine extended-release formulation. *Aliment. Pharmacol. Ther.*, **17**, 395–402 (2003).
- 14) Matthis AL, Zhang B, Denson LA, Yacyshyn BR, Aihara E, Montrose MH. Importance of the evaluation of *N*-acetyltransferase enzyme activity prior to 5-aminosalicylic acid medication for ulcerative colitis. *Inflamm. Bowel Dis.*, **22**, 1793–1802 (2016).
- 15) Vree TB, Dammers E, Exler PS, Sorgel F, Bondesen S, Maes RA. Liver and gut mucosa acetylation of mesalazine in healthy volunteers. *Int. J. Clin. Pharmacol. Ther.*, **38**, 514–522 (2000).
- 16) Jennne JW. Partial purification and properties of the isoniazid transacetylase in human liver. Its relationship to the acetylation of *p*-aminosalicylic acid. *J. Clin. Invest.*, **44**, 1992–2002 (1965).
- 17) Weber WW, Hein DW. *N*-Acetylation pharmacogenetics. *Pharmacol. Rev.*, **37**, 25–79 (1985).
- 18) Hickman D, Pope J, Patil SD, Fakis G, Smelt V, Stanley LA, Payton M, Unadkat JD, Sim E. Expression of arylamine *N*-acetyltransferase in human intestine. *Gut*, **42**, 402–409 (1998).
- 19) Vatsis KP, Weber WW, Bell DA, Dupret JM, Evans DA, Grant DM, Hein DW, Lin HJ, Meyer UA, Relling MV, Sim E, Suzuki T, Yamazoe Y. Nomenclature for *N*-acetyltransferases. *Pharmacogenetics*, **5**, 1–17 (1995).
- 20) Sekine A, Saito S, Iida A, Mitsunobu Y, Higuchi S, Harigae S, Nakamura Y. Identification of single-nucleotide polymorphisms (SNPs) of human *N*-acetyltransferase genes NAT1, NAT2, AANAT, ARD1 and L1CAM in the Japanese population. *J. Hum. Genet.*, **46**, 314–319 (2001).
- 21) Fallingborg J, Christensen LA, Jacobsen BA, Rasmussen SN. Very low intraluminal colonic pH in patients with active ulcerative colitis. *Dig. Dis. Sci.*, **38**, 1989–1993 (1993).