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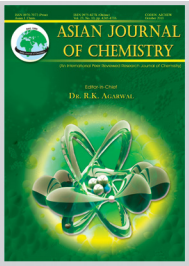

5-Aminosalicylic Acid Released from Mesalazine Tablet-Comparison of Pharmacokinetic Parameters between Japanese Patients with Ulcerative Colitis and Healthy Adults

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A slow release formulation of mesalazine (5-aminosalicylic acid, 5-ASA) is commonly used for ulcerative colitis. We aimed to compare the pharmacokinetic profile of 5-ASA in Japanese patients with ulcerative colitis (UC patients) and healthy adults received 2 g single dose of mesalazine tablets. Main outcome measures were plasma and urinary concentrations of 5-ASA and its major metabolite *N*-acetyl-5-aminosalicylic acid (Ac-5-ASA) up to 12 h post-dose. 5-Aminosalicylic acid maximum plasma concentration (C_{max}) and area under the plasma concentration-time curve ($AUC_{0\text{-}inf}$) in ulcerative colitis patients: 5197 ± 3345 ng/mL and 24333 ± 11542 ng h/mL, respectively; in healthy adults: 7190 ± 5093 ng/mL and 23890 ± 12684 ng h/mL, respectively. Total urinary excretion rate of 5-ASA and Ac-5-ASA: 25.2 ± 6.8 % in ulcerative colitis patients; 18.0 ± 9.9 % in healthy adults. The pharmacokinetic profile of 5-ASA was comparable between Japanese ulcerative colitis patients and healthy adults.

Key Words: 5-Aminosalicylic acid, Pentasa® tablet, Pharmacokinetics, Ulcerative colitis, Healthy adults.

INTRODUCTION

Mesalazine (5-aminosalicylic acid, 5-ASA), is commonly used to induce remission and as maintenance therapy for ulcerative colitis[1]. Reported pharmacological effects of 5-ASA on ulcerative colitis include localized elimination of active oxygen at inflammatory sites in the mucosa of the large intestine[2] and suppression of leukotriene B4 biosynthesis[3]. When intact 5-ASA is orally administered, most of the dose is rapidly absorbed from the small intestine[4]. Therefore, controlled release formulations of 5-ASA (time-dependent controlled release formulation and pH-dependent controlled release formulation) have been developed to enhance the therapeutic effects of the drug on ulcerative colitis. Although there are quite differences between 5-ASA formulations in release patterns, there are no differences in therapeutic effects.

A slow-release formulation of mesalazine (Pentasa® Tablet), a formulation of 5-ASA, is coated with a multi-pore film of ethylcellulose that time-dependently releases 5-ASA into the gastrointestinal tract at pHs 2 to 6, but is enhanced at pHs above 6[5-9]. Results of pharmacoscintigraphy studies have shown the release of 5-ASA from this orally administered mesalazine tablet in all areas of the small and large intestine[10]. Orally administered 5-ASA is converted to *N*-acetyl-5-aminosalicylic acid (Ac-5-ASA) by *N*-acetyltransferase present in the gastrointestinal mucosa and the liver and is excreted into the urine[11].

Results of clinical studies reported to date in European subjects indicate that the pharmacokinetic profile of 5-ASA released from mesalazine tablets is similar between healthy adults and ulcerative colitis patients and between ulcerative colitis patients in the active phase (active ulcerative colitis patients) and those with ulcerative colitis in remission (quiescent ulcerative colitis patients). Christensen *et al.*[12] investigated mesalazine tablet (5-ASA 2000 mg/day) orally administered repeatedly for 7 days to 13 healthy adults. They reported that the mean steady state plasma concentration after 7 days was 521 ng/mL for 5-ASA and 1405 ng/mL for Ac-5-ASA and that the mean total urinary excretion rate (5-ASA and Ac-5-ASA) on days 6 and 7 of dosing was 30.5 %. Laursen *et al.*[13] investigated a 7-day repeated administration of mesalazine tablet (5-ASA 2000 mg/day) in 14 quiescent ulcerative colitis patients. The plasma concentration was 398 ng/mL for 5-ASA and 1581 ng/mL for Ac-5-ASA and the mean total urinary excretion rate was 36 %. In addition, Rijk *et al.*[14] evaluated a 5-week repeated oral administration of mesalazine tablet (5-ASA 1500 mg/day) in 20 patients with inflammatory bowel disease (IBD) (five ulcerative colitis patients, 15 patients with Crohn's disease) who were divided into subgroups of 10 patients with signs of diarrhoea (one ulcerative colitis patient, nine patients with Crohn's disease) and 10 patients without signs of diarrhoea (four ulcerative colitis patients, six patients with Crohn's disease).

This study reported that the mean total urinary excretion rate (5-ASA and Ac-5-ASA on days 6 and 7 in each week), was 25.1 % and 22.7 %, respectively, for each subgroup.

The present study compared the pharmacokinetic profile of 5-ASA in Japanese healthy adults and ulcerative colitis patients following oral administration of 2000 mg single dose of slow release mesalazine tablets (4 × 500 mg tablets). A 2000 mg single dose corresponds to the standard dosage and administration for the treatment of ulcerative colitis patients. While from an exploratory point of view, the transition of 5-ASA and Ac-5-ASA concentration in the blood and the pharmacokinetic profile of 5-ASA between active and quiescent ulcerative colitis patients were considered.

EXPERIMENTAL

Study population: Healthy Japanese adults without history of drug-hypersensitivity and ulcerative colitis patients who gave their informed consent to participate in the study were enrolled. The disease phase of ulcerative colitis was determined based on clinical signs, endoscopic findings and histopathological findings. To exclude effects of the study drug on pharmacokinetic measurements, ulcerative colitis patients were not allowed to administer any 5-ASA and salazosulfapyridine from 12 h before the start of treatment with the study drug. The study was conducted in compliance with the ethical principles according to the Declaration of Helsinki and following approval by the Institutional Review Board of the study site.

Study design: Pharmacokinetic study in healthy adults. 4 Mesalazine tablets (4 × 500 mg tablets; 5-ASA 2000 mg) were orally administered with 180 mL of water, 0.5 h after breakfast. Venous blood (7 mL/time point) was sampled from the forearm vein of the subject into heparinized sampling tubes before dosing, at 0.5 h min and at 1, 2, 3, 4, 6, 8 and 12 h post-dose. Immediately after sampling, the blood was centrifuged for 10 min at 4 ºC, 3000 rpm and the plasma obtained was divided into two polypropylene sample stock tubes and stored frozen at -20 ºC or lower until analyzed. Urine samples taken during the periods of 12-0 h pre-dose, 0-6 h post-dose and 6-12 h post-dose were separately collected in polyethylene bottles and stored at 4 ºC or lower. The cumulated urine samples were weighed and the specific gravity measured. The samples were then divided into two polypropylene sample stock tubes (10 mL/tube) and stored frozen at -20 ºC or lower until analyzed.

Before the start of treatment, laboratory tests (haematology, blood biochemistry and urinalysis) and measurement of vital signs (body temperature and heart rate) were performed. ECG (12-lead) was examined before dosing and at 4 and 12 h post-dose. Holter ECG (1-lead) was examined within 24 h before dosing to 4 h post-dose. The presence or absence of adverse events (AEs) was confirmed by interview before dosing and at 1 h post-dose. The subjects were provided with a meal at 3.5 and 9.5 h post-dose.

Pharmacokinetic study in patients with ulcerative colitis: 4 Mesalazine tablets (4 × 500 mg tablets; 5-ASA 2000 mg) were orally administered with 150 mL of water, 0.5 h after breakfast. Venous blood (9 mL/time point) was sampled from the fore arm vein of the patients into heparinized blood sampling tubes before dosing and at 1, 2, 3, 4, 5, 6 and 12 h post-dose. Immediately after sampling, the blood was centrifuged for 10 min at 4 ºC, 3000 rpm and the plasma obtained was divided into two polypropylene sample stock tubes and stored frozen at -20 ºC or lower until analyzed. Urine samples taken pre-dosing were divided into two polypropylene sample stock tubes and stored frozen at -20 ºC or lower until analyzed. Urine samples taken during the period of 0-6 h and 6-12 h post-dose were separately collected in polyethylene bottles and stored at 4 ºC or lower. The cumulated urine samples were weighed and the specific gravity was measured. The samples were then divided into two polypropylene sample stock tubes (10 mL/tube) and stored frozen at -20 ºC or lower until analyzed.

Before dosing and at 12 h post-dose, laboratory tests (haematology, blood biochemistry and urinalysis) were performed and vital signs (body temperature and heart rate) were measured before dosing and at 2, 4, 8 and 12 h post-dose. The presence or absence of adverse events was confirmed by interview before dosing and at 4 and 12 h post-dose. The patients were provided with a meal at 4 and 8 h post-dose.

Quantitative analysis of 5-ASA and Ac-5-ASA in plasma: Distilled water (500 µL) and anhydrous propionic acid (10 µL) were added to 500 µL of plasma. After mixing, the mixture was left in the dark at room temperature for 10 min. Then, 100 µL of 25 % phosphate was added followed by 6 mL of *tert*-butyl methyl ether. After mixing by shaking, the mixture was centrifuged (3000 rpm, 5 min) and 4 mL of the organic layer was evaporated. The residue was dissolved in 400 µL of 10 mmo/L sodium phosphate buffer (pH 2.8) and 20 µL was injected into the high performance liquid chromatography (HPLC): column: Inertsil ODS-2, 5 µm (250 × 4.6 mm); column temperature: 45 ºC; mobile phase: methanol/10mmo/L sodium phosphate buffer (pH 2.8) = 3/7 (vol/vol); velocity: 1.0 mL/min; wave length of detection: excitation wave length 305 nm/fluorescent wave length 418 nm. Limit of detection for both 5-ASA and Ac-5-ASA was 10 ng/mL.

Quantitative analysis of 5-ASA and Ac-5-ASA in urine: Urine (50 µL) was diluted with 5 mL of 10 mmo/L sodium phosphate buffer (pH 2.5) and a volume of 1 mL was taken. After adding 10 µL of anhydrous propionic acid and mixing, the mixture was left in the dark at room temperature for 10 mins. After adding 8 mL of *tert*-butyl methyl ether and mixing by shaking, the mixture was centrifuged (3000 rpm, 5 min) and 4 mL of the organic layer was evaporated. The residue was dissolved in 1.5 mL of methanol/10 mmo/L sodium phosphate buffer (pH 2.5) = 3/7 (vol/vol) and a volume of 50 µL was injected into the HPLC: column: Inertsil ODS-2, 5 µm (250 × 4.6 mm); column temperature: 45 ºC; mobile phase: methanol/10 mmol/L sodium phosphate buffer (pH2.8) = 3/7 (vol/vol); velocity: 1.0 mL/min; wave length of detection: excitation wave length 315 nm/fluorescent wave length 418 nm. Limit of detection for both 5-ASA and Ac-5-ASA was 10 ng/mL.

Pharmacokinetic analysis: The elimination rate constant (k_{el}) was determined by linear regression for the linear part of the elimination phase of the plasma concentration (logarithmic value)-time curve using the least-squares method. The area under the plasma concentration-time curve up to the final sampling time point t (AUC_{0-t}) was calculated by the trapezoidal

method. $AUC_{0\text{-}inf}$ was calculated by adding C_t/k_{el} (C_t: plasma concentration at the final sampling time point) to $AUC_{0\text{-}t}$. The apparent systemic clearance (CL_{tot}/F) was calculated from dose level/$AUC_{0\text{-}inf}$. The urinary excretion rate ($Ae_{0\text{-}t}$) was calculated from urinary excretion/dose. To calculate $Ae_{0\text{-}t}$ for Ac-5-ASA, 2.5488 g (molecular weight of Ac-5-ASA (195.17)/molecular weight of 5-ASA (153.14) × 2) was used as the dose.

Sample size and statistical methods: The T test was used to compare mean values of the pharmacokinetic parameters between the healthy adults and patients with ulcerative colitis. Two-tailed 5 % was used as the significance level.

RESULTS AND DISCUSSION

Subjects disposition and characteristics: The baseline characteristics of the subjects are shown in Tables 1 and 2. All the subjects were Japanese adult males. The study comprised six healthy adults without history of drug-hypersensitivity (aged 24.3 ± 3.3 years, 63.8 ± 7.6 kg) and ten ulcerative colitis patients (aged 41.6 ± 12.7 years, 63.2 ± 9.8 kg). The baseline characteristics of active ulcerative colitis patients (aged 39.0 ± 9.3 years, 59.5 ± 7.7 kg) and four quiescent ulcerative colitis patients with (aged 45.5 ± 17.6 years, 68.8 ± 11.0 kg).

TABLE-1
BASELINE CHARACTERISTICS OF HEALTHY ADULTS AND UC PATIENTS

	Healthy adults (n = 6)	UC patients (n = 10)
Age, years, mean ± SD	24.3 ± 3.3	41.6 ± 12.7
Weight, kg, mean ± SD	63.8 ± 7.6	63.2 ± 9.8

TABLE-2
BASELINE CHARACTERISTICS OF ACTIVE AND QUIESCENT UC PATIENTS

	Active UC patients (n = 6)	Quiescent UC patients (n = 4)
Age, years, mean ± SD	39.0 ± 9.3	45.5 ± 17.6
Weight, kg, mean ± SD	59.5 ± 7.7	68.8 ± 11.0
Prior dose of 5-ASA (mg/kg/day), mean ± SD	2000 ± 387*	1875 ± 433*

*All subjects had been continuously taking mesalazine tablet up to the previous day.

Pharmacokinetic parameters in healthy adults and ulcerative colitis patients: Pharmacokinetic parameters of 5-ASA and its major metabolite Ac-5-ASA in the six healthy adults as well as the 10 ulcerative colitis patients are shown in Table-3. Their corresponding log of plasma concentration-time curves are shown in Figs. 1 and 2. Plasma concentrations of 5-ASA peaked at 2-3 h post-dose in all groups, followed by a decrease to around the baseline level at 12 h post-dose. Plasma concentrations of Ac-5-ASA remained high compared with those of 5-ASA. The mean C_{max} values of 5-ASA and Ac-5-ASA in ulcerative colitis patients tended to be low compared with those of the healthy adults, however, there was no significant difference between the two groups. The mean values of $AUC_{0\text{-}inf}$ for 5-ASA were comparable between the healthy adults and ulcerative colitis patients and there was no difference between the healthy adults and ulcerative colitis patients in the mean values of $AUC_{0\text{-}inf}$ for Ac-5-ASA. The total urinary excretion rate of 5-ASA and Ac-5-ASA in ulcerative colitis patients up to 12 h post-dose was 25.2 ± 6.8%, which tended to be slightly higher than that in the healthy adults (18.0 ± 9.9 %), however, no significant difference was observed.

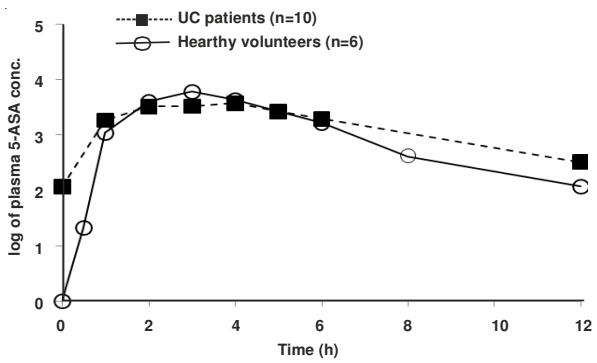

Fig. 1. Mean plasma 5-ASA concentrations in six healthy adults (O) and 10 patients with UC (■) following oral administration of mesalazine tablet containing 2000 mg of 5-ASA

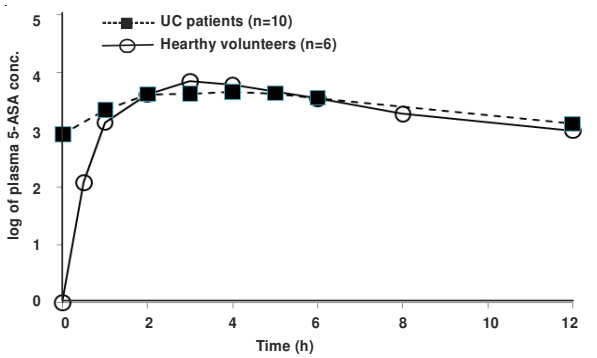

Fig. 2. Mean plasma Ac-5-ASA concentrations in six healthy adults (O) and 10 patients with UC (■) following oral administration of mesalazine tablet containing 2000 mg of 5-ASA

TABLE-3
PHARMACOKINETIC PARAMETERS FOR 5-ASA AND Ac-5-ASA IN UC PATIENTS AND HEALTHY ADULTS FOLLOWING ORAL ADMINISTRATION OF MESALAZINE TABLET (5-ASA 2000 mg)

	C_{max} (ng/mL)	t_{max} (h)	$AUC_{0\text{-}inf}$ (ng h/mL)	CL_{tot}/F (L/h)	$Ae_{0\text{-}12}$ (%)
Healthy adults (n = 6)					
5-ASA	7190 ± 5093	2.8 ± 0.8	23890 ± 12684	116 ± 77.7	3.3 ± 3.1
Ac-5-ASA	7676 ± 4671	3.0 ± 0.9	49942 ± 19265	60.2 ± 29.0	14.7 ± 7.1
UC patients (n = 10)					
5-ASA	5197 ± 3345	2.5 ± 1.4	24333 ± 11542	100 ± 50.0	6.3 ± 3.2
Ac-5-ASA	5615 ± 3144	2.7 ± 1.1	44808 ± 19128	52.1 ± 22.2	18.8 ± 4.2
Statistical analysis (t test) of the difference between patients with UC and healthy adults					
5-ASA	0.358	0.609	0.944	0.621	0.090
Ac-5-ASA	0.307	0.572	0.612	0.539	0.160

TABLE-4
PHARMACOKINETIC PARAMETERS FOR 5-ASA AND Ac-5-ASA IN ACTIVE UC PATIENTS AND QUIESCENT UC PATIENTS FOLLOWING ORAL ADMINISTRATION OF PENTASA® TABLET (5-ASA 2000 mg)

	C_{max} (ng/mL)	t_{max} (h)	$AUC_{0\text{-}inf}$ (ng h/mL)	CL_{tot}/F (l/h)	$Ae_{0\text{-}12}$ (%)
Active UC patients (n = 6)					
5-ASA	4183 ± 2045	2.8 ± 1.5	23553 ± 10129	102 ± 57.1	7.0 ± 4.0
Ac-5-ASA	4648 ± 1914	2.7 ± 0.8	41910 ± 13516	53.7 ± 23.9	19.4 ± 4.7
Quiescent UC patients (n = 4)					
5-ASA	6718 ± 4632	2.0 ± 1.4	25504 ± 15020	96.5 ± 43.8	5.2 ± 1.7
Ac-5-ASA	7065 ± 4345	2.8 ± 1.5	49156 ± 27408	49.6 ± 22.6	18.0 ± 3.7

Their corresponding log of plasma concentration-time curves in the active ulcerative colitis and quiescent ulcerative colitis patients are shown in Figs. 3 and 4. Pharmacokinetic parameters of 5-ASA and its major metabolite Ac-5-ASA in the active ulcerative colitis and quiescent ulcerative colitis patients are shown in Table-4.

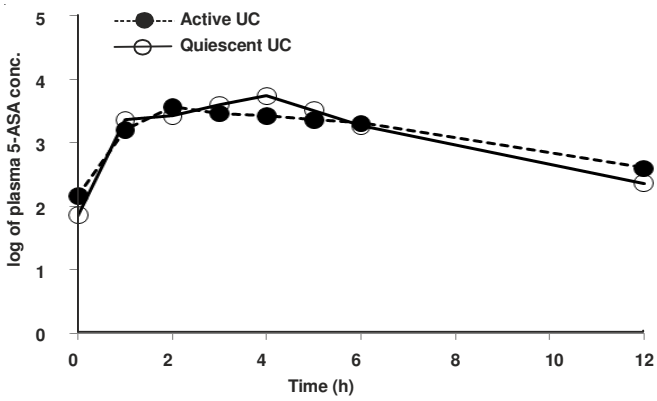

Fig. 3. Mean plasma 5-ASA concentrations in 4 quiescent UC patients (O) and 6 active UC patients (●) following oral administration of mesalazine tablet containing 2000 mg of 5-ASA

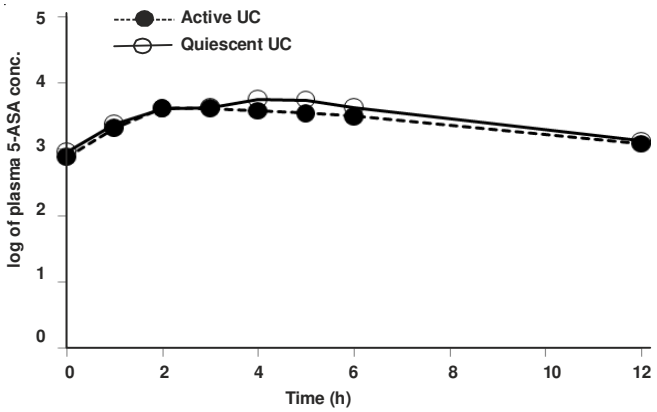

Fig. 4. Mean plasma Ac-5-ASA concentrations in 4 quiescent UC patients (O) and 6 active UC patients (●) following oral administration of mesalazine tablet containing 2000 mg of 5-ASA

Adverse events: Adverse events observed during the study period included one case of hard faeces in a healthy adult and one case of inflammation of the upper respiratory tract in a ulcerative colitis patient. Both of these events were mild in severity with no relationship to the study drug.

Mesalazine(5-ASA) acts locally after absorption from ileal and/or colonic mucosa, not after oral absorption. After 5-ASA is absorbed, it is rapidly metabolized to Ac-5-ASA and primarily excreted in the urine[12]. The optimal concentration of 5-ASA for clinical effect has not been demonstrated, but it might depend on the severity and/or extent of inflammation. Hanauer *et al.*[15] demonstrated that treatment with Pentasa 1, 2 and 4 g/day for patients with mild to moderately active ulcerative colitis shows better clinical effects with increasing dose. This result suggests that the therapeutic effect is probably related to the local concentration of 5-ASA in the colonic mucosa.

As there are very large inter individual variations in human bowel movement, blood concentrations of 5-ASA and Ac-5-ASA also show large variation after oral administration. There is concern that the efficacy and safety of 5-ASA will be affected. It has been suggested that when accelerated intestinal transit time is induced, as with diarrhoea, a typical symptom in patients with IBD, there will be an effect on dispersion in many mesalazine formulations[16,17]. Furthermore, the absorption of delayed release formulations of 5-ASA coated with a pH-sensitive acrylic based resin (Eudragit L and Eudragit S) seems more influenced by variations in gastric pH, as has been shown in several manuscripts[5-9,18]. It has been otherwise demonstrated that Pentasa® Tablet are a time-dependent slow-release formulation little influenced by variations in human bowel movement[19].

Some pharmacokinetic study results demonstrated no significant difference between healthy adults and patients with IBD.[4,20] in only western patients and healthy adults. On the other hand, no information has been obtained in Asian patients with IBD. This study confirmed about the pharmacokinetic parameters in ulcerative colitis patients and demonstrated that there are no significant differences in the pharmacokinetic parameters (C_{max}, t_{max}, $AUC_{0\text{-}inf}$, CL_{tot}/F and $Ae_{0\text{-}12}$) of 5-ASA and Ac-5-ASA between Japanese healthy adults and ulcerative colitis patients who are orally treated with mesalazine tablet as the same as western subjects. In addition, there seems no significant differences in the transition of 5-ASA and Ac-5-ASA concentration in the blood and the pharmacokinetic profiles between active ulcerative colitis patients and quiescent ulcerative colitis patients. These results suggest that signs of diarrhoea and intestinal mucosal disorders such as erosion that are observed in ulcerative colitis patients have no effect on plasma concentration or mean urinary excretion rate of 5-ASA.

Furthermore, the total urinary excretion rate of 5-ASA and Ac-5-ASA in Japanese healthy adults proved to be comparable to that seen previously in western subjects. The total urinary excretion rate in European and American healthy adults following oral administration of mesalazine tablet containing 250, 500 and 1000 mg of 5-ASA has been reported to be 18 % (48 h post-dose)[10], 20 % (24 h post-dose)[11] and 29 % (96 h

post-dose)[4] respectively. The total urinary excretion rate in Japanese healthy adults seen in the present study (18 %, 12 hours post-dose) was approximate to these values, suggesting that the pharmacokinetics of 5-ASA in Japanese and Western subjects is similar.

In addition, it was showed that C_{max} and $AUC_{0\text{-}inf}$ values of 5-ASA in active ulcerative colitis patients don't exceed those values in healthy adults (Tables 3 and 4), suggesting that increase in absorption of 5-ASA in active ulcerative colitis patients due to injury of gastrointestinal mucosa is unlikely.

Conclusion

The pharmacokinetic profile of 5-ASA released from mesalazine tablet proved to be comparable between Japanese ulcerative colitis patients and healthy adults. The total urinary excretion rate of 5-ASA and Ac-5-ASA in Japanese healthy adults was approximate to that seen in western subjects, suggesting that there is no ethnic difference between Japanese and Europeans and Americans in terms of the pharmacokinetics of 5-ASA.

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