Comparative Absorption of 5-Aminosalicylic Acid (5-ASA) after Administration of a 5-ASA Enema and Salazosulfapyridine (SASP) after an SASP Suppository in Japanese Volunteers

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Salazosulfapyridine (SASP) is widely used orally and rectally in the treatment of ulcerative colitis. SASP is mainly metabolized by hydrolysis and the main active metabolite, 5-aminosalicylic acid (5-ASA), has an antiinflammatory effect. In the present study, we prepared suppositories containing 6.5 mmol of SASP and an enema containing 6.5 mmol of 5-ASA. We measured the concentrations of SASP and its various metabolites, 5-ASA, sulfapyridine (SP), acetylated metabolite of SP (Ac-SP), and N-acetyl-5-ASA (Ac-5-ASA), in the serum and urine after a single administration of each preparation to healthy male volunteers. When the SASP suppository was administered, the maximum concentration (Cmax) of SASP and Ac-5-ASA was 2.5±0.4 and 0.5±0.2 μM and the time to Cmax (Tmax) was 5 and 12 h, respectively. The Cmax value of SP, which causes side effects, was one-half of that of the parent compound. No 5-ASA in the serum was observed. When the 5-ASA enema was administered, Cmax and Tmax values of 5-ASA and Ac-5-ASA were 5.8±2.0 and 13.3±3.6 μM and 1 and 7 h, respectively. The area under the serum concentration–time curve (AUC) of SASP was 27.4±4.8 μg·h·M, a finding similar to that of 5-ASA after the administration of the 5-ASA enema (29.4±11.1 μg·h·M). The percentage of urinary recovery of SASP 24 h after administration of the SASP suppository was approximately 0.2%. These results indicate that SASP administered rectally is almost completely hydrolyzed in the colon and that 5-ASA is partially absorbed from the small intestine in unchanged form. On the other hand, approximately 0.3% of 5-ASA was recovered in the urine in unchanged form after the administration of the 5-ASA enema, whereas the urinary recovery of Ac-5-ASA was more than 10%. The present findings suggest that 5-ASA has favorable absorptive properties and can be expected to have systemic action after rectal administration of a 5-ASA enema.

Key words 5-aminosalicylic acid; salazosulfapyridine; enema; suppository; pharmacokinetic

Ulcerative colitis is a nonspecific inflammation of unknown etiology, which infiltrates the colon from the rectum. Salazosulfapyridine (SASP), an antiinflammatory agent, is used clinically to treat ulcerative colitis as a suppository. In Japan, the use of SASP preparations in the treatment of ulcerative colitis is mainly limited to oral administration, and rectal administration is rare. The main product of the azo-coupling of 5-aminosalicylic acid (5-ASA) and sulfapyridine (SP), is administered orally, the azo-coupling is hydrolyzed by colonic bacterial enzymes into 5-ASA and SP in an area extending from the end of the ileum through the colon, and the components are acetylated to N-acetyl-5-ASA (Ac-5ASA) and acetyl-SP (Ac-SP), respectively (Fig. 1). One of these metabolites, 5-ASA, has an antiinflammatory effect on the colonic mucosa, and SP appears to cause side effects. Several mechanisms have been proposed to explain the effect of 5-ASA, including the inhibition of prostaglandin synthesis, inhibition of arachidonic acid metabolism, and an inhibitory effect on leukocyte chemotaxis. Direct administration of 5-ASA into the inflammatory sites might be useful for the treatment of ulcerative colitis. A 5-ASA enema preparation is currently used in the U.S.A. However, clinical pharmacokinetic studies of 5-ASA in the enema form have been limited in Japan, although there have been several studies abroad on the pharmacokinetics and bioavailability of SASP and 5-ASA preparations.

On the other hand, it is well known that genetic polymorphism occurs in N-acetyltransferase: the frequency of the poor metabolizer with deficient activity is about 50% in Caucasians and 10% in Japanese. This genetic polymorphism might lead to differences in the pharmacokinetics of SASP and 5-ASA between Caucasians and Japanese. Therefore it is necessary to investigate the pharmacokinetic characteristics of SASP and 5-ASA in a Japanese population.

In the present study, we prepared an SASP suppository containing 6.5 mmol of SASP.

**Fig. 1. Proposed Metabolic Pathway of Salazosulfapyridine after Oral Administration**

SASP, salazosulfapyridine; SP, sulfapyridine; 5-ASA, 5-aminosalicylic acid; Acetyl-5-ASA, acetylated 5-aminosalicylic acid.

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and a 5-ASA enema and evaluated the comparative pharmacokinetics of SASP and 5-ASA in healthy Japanese volunteers.

MATERIALS, VOLUNTEERS AND METHODS

Chemicals SP and 5-ASA were purchased from Tokyo Kasei (Tokyo, Japan). SASP was obtained from Welfide Corp. (Osaka, Japan). Ac-SP was synthesized by acetylation of SP with anhydrous acetic acid and then recrystallized with 70% dimethylsulfoxide (DMSO). Ac-5-ASA was synthesized as described by Peppercorn.15) 5-ASA was acetylated by anhydrous acetic acid, and the O-acetyl groups were hydrolyzed in an alkaline solution and then recrystallized with ethanol. The purity of each synthesized compound was confirmed by thin-layer chromatography and mass-spectrometry. All other chemicals used were commercially available and of analytical grade.

Preparation of the 5-ASA Enema and SASP Suppository The 5-ASA enema was prepared based on the method reported by Azad and colleagues.9) The enema consisted of 1 g of 5-ASA, 0.05 g of ascorbic acid as an antioxidant, and 0.18 g of methylparaben and 0.02 g of propylparaben as stabilizers in a volume of 100 ml. The enema was prepared immediately before administration. The SASP suppositories weighed about 12.5 g and contained a dose equivalent to SASP 2.59 g. They were prepared using Witepsol H15 (Dynamit Nobel Chemicals, Oberlar, Germany) which is a mixture of mono-, di-, and triglycerides of saturated fatty acids with a melting range of 33—36 °C. SASP was suspended in a suppository base at 45 °C, and subjected to a moulding procedure. The suppositories were stored at 4 °C and used within 1 week of preparation.

Volunteers and Bioavailability Experiments Three healthy male volunteers, between 20 and 26 years of age (mean, 23.3 years), participated in this study. All subjects were judged to be healthy on the basis of their medical histories, physical examinations, and laboratory tests of blood and urine samples before the study. Informed consent was obtained from each subject after he had been given a full explanation of the procedures.

Drug trials were performed in a crossover manner with a 1-week interval for the respective preparations. During the first phase, all subjects received orally 2000 ml of a colonic fiber laxative that had been prepared as described by Davis et al.,15) over a period of 2 h to empty their bowels. One hour after their bowels had been completely emptied, the subjects received a single rectal administration of a SASP suppository. Blood samples (about 10 ml) were collected 1, 3, 5, 7, 12, and 24 h later in plain tubes and then immediately centrifuged to yield serum. Urine samples were collected every 2 h over a period of 24 h and their volumes were measured. The serum and 10 ml of each urine sample were stored at −40 °C until analysis. In the second phase, all subjects received a 5-ASA enema one week after the administration of the SASP suppository. Blood and urine samples were collected as described above.

Drug Analysis Concentrations of SASP, 5-ASA, and their metabolites in the serum or the urine were measured by high-performance liquid chromatography (HPLC) as reported by Fischer et al.,16) and Shaw et al.17) The HPLC system includes an LC-6A liquid pump, an RF-550 spectrofluorometric detector, and an SIL-6A autoinjector (Shimadzu LC-6A system; Shimadzu Co., Kyoto, Japan). The column was a Cosmosil 5C18 column (6 × 250 mm, Nacalai Tesque, Kyoto, Japan). The mobile phase was 50 mM phosphate buffer–methanol (3:1, vol/vol) containing 1% trifluoroacetic acid. The mobile phase was employed at a flow rate of 1.0 ml/min. Elution was carried out at 35 °C, and the effluent was monitored at 310 nm (excitation) and 455 nm (emission) for SASP or 410 nm (emission) for other compounds. The coefficients of variation for each measurement were less than 7%.

Data Analysis The serum concentration–time data for each compound for each subject in each study phase were analyzed on the basis of noncompartmental methods. The maximum concentration (Cmax) and the time to Cmax (Tmax) were obtained from the observed data. The area under the serum concentration–time curve (AUC) was calculated by the trapezoidal rule for values up to the last measured concentration in the serum. The recovery of each metabolite or unchanged compound in the urine is represented as a percentage of the total excretion.

Statistical Analysis The means of all data are presented with their standard error (mean±S.E.M.). Statistical analysis was performed using the non-paired Student’s t-test, and a p value of 0.05 or less was considered to be significant.

RESULTS

In this study, administration of the SASP suppository or 5-ASA enema was well tolerated, with no side effects experienced by any of the three volunteers following the single administration of each preparation.

To compare the fate of 5-ASA after administration of the 5-ASA enema with that of SASP after administration of the SASP suppository, serum concentrations of the parent compound and its metabolites 5-ASA and Ac-5-ASA were determined. The mean serum concentration–time curves for SASP and its metabolites after a single administration of the SASP suppository are shown in Fig. 2. The corresponding pharma-
Table 1. Pharmacokinetic Parameters of SASP and Its Metabolites after a Single Administration of an SASP Suppository

<table>
<thead>
<tr>
<th>Compound</th>
<th>AUC (µM·h)</th>
<th>T_max (h)</th>
<th>C_max (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SASP</td>
<td>27.4±4.8</td>
<td>5.0±0.0</td>
<td>2.5±0.4</td>
</tr>
<tr>
<td>SP</td>
<td>19.3±9.6</td>
<td>5.0±0.0</td>
<td>1.2±0.4</td>
</tr>
<tr>
<td>Ac-SASP</td>
<td>23.0±6.2</td>
<td>12.0±0.0</td>
<td>1.0±0.3</td>
</tr>
<tr>
<td>Ac-5-ASA</td>
<td>11.3±4.1</td>
<td>12.0±0.0</td>
<td>0.5±0.2</td>
</tr>
</tbody>
</table>

SASP, salazosulfapyridine; SP, sulfapyridine; Ac-SASP, acetylated sulfapyridine; Ac-5-ASA, acetylated 5-aminosalicylic acid. a) Significantly different from SASP (p<0.05). b) Significantly different from SP (p<0.05). Each value represents the mean±S.E.M. (n=3).

Table 2. Pharmacokinetic Parameters of 5-ASA and Its Metabolite after a Single Administration of a 5-ASA Enema

<table>
<thead>
<tr>
<th>Compound</th>
<th>AUC (µM·h)</th>
<th>T_max (h)</th>
<th>C_max (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-ASA</td>
<td>29.4±11.1</td>
<td>1.0±0.0</td>
<td>5.8±2.0</td>
</tr>
<tr>
<td>Ac-5-ASA</td>
<td>73.3±32.8</td>
<td>7.0±0.0</td>
<td>13.3±3.6</td>
</tr>
</tbody>
</table>

5-ASA, 5-aminosalicylic acid; Ac-5-ASA, acetylated 5-aminosalicylic acid. a) Significantly different from 5-ASA (p<0.05). Each value represents the mean±S.E.M. (n=3).

DISCUSSION

SASP administered orally is partially absorbed from the small intestine, but most of it is hydrolyzed into SP and 5-ASA in the colon by the action of colonic bacterial enzymes.3) It has been reported that SP is easily absorbed, whereas only approximately one-third of 5-ASA produced in the colon is absorbed and the rest is excreted in feces.13) The SP absorbed is acetylated or hydrolyzed in the liver or in various tissues and further conjugated with glucuronic acid, and is excreted in the urine.4,12) The present study focused on the pharmacokinetics of 5-ASA after administration of a 5-ASA enema and compared with those of SASP after administration of an SASP suppository to Japanese volunteers.

In general, rectal administration of drugs as suppositories results in somewhat lower bioavailability than oral administration because of the small quantity of fluid in the rectum and the smaller absorption surface area, and because there is no strong muscular action such as peristalsis. However, a drug in an enema preparation is known to be more rapidly absorbed than that in suppository form. In the present study, we found that 5-ASA administered as an enema was absorbed rapidly, whereas SASP administered as a suppository was absorbed slowly, with a T_max value of 5 h, confirming that the absorption rate of a drug as an enema is faster than that as a suppository. It is likely that after the base has melted at the temperature in the rectum, SASP particles are in a state

![Fig. 3. Serum Concentration–Time Profiles for 5-Aminosalicylic Acid (5-ASA) and Acetylated 5-ASA (Ac-5-ASA) after a Single Rectal Administration of a 5-ASA Enema](Image)
of suspension and dissolved in the secreted fluid as a prestep to absorption.

The current study revealed that the percentage of SASP and its metabolites excreted into the urine is only about 0.6% of the dose administered during the 24 h after the administration of the SASP suppository and that a small amount of SASP administered rectally is absorbed in unchanged form (Tables 2, 3). These results are, in part, supported by those in previous studies.13) Assuming that the absorbed fraction of 5-ASA is 10%, since the half-life of Ac-5-ASA has been reported to be 6 to 9 h,11,13) the major metabolite Ac-5-ASA was detected in the serum after the administration of the SASP suppository, while 5-ASA was not detected, suggesting that 5-ASA absorbed from the small intestine is rapidly acetylated to Ac-5-ASA in the body. Klotz11) has suggested that Ac-5-ASA possesses pharmacological activity. The AUC value of Ac-5-ASA after the administration of the 5-ASA enema was 6-fold that of the SASP suppository (Tables 1, 2). These results suggest that the 5-ASA enema has much stronger antiinflammatory activity in the body than the SASP suppository. After the administration of the 5-ASA enema, a secondary increase in the concentration of Ac-5-ASA in the serum was observed, although the differences between concentrations of Ac-5-ASA at 5 and 7 h after administration failed to reach the 5% level of statistical significance. The reason is not clear at present, although it may be explained by enterohepatic circulation of 5-ASA. It is likely that 5-ASA produced by hydrolysis in the colon, which probably acts topically at inflammatory sites in the mucosa, is excreted into feces after the administration of the enema form. On the other hand, the percentage of 5-ASA and Ac-5-ASA excreted into the urine after the administration of the enema was approximately 0.3% and 11%, respectively. These results differed from the clinical findings reported by Klotz and Maier,13) who demonstrated that about one-third of the dose of 5-ASA is recovered in the urine, almost entirely as Ac-5-ASA. The reason for discrepancy between their results and ours remains to be elucidated, although it might be due to differences in pharmacokinetic behavior between healthy volunteers and patients with inflammatory bowel disease and/or the genetic polymorphism of N-acetyltransferase. There is a possibility that our urine collection time was inadequate, since the half-life of Ac-5-ASA has been reported to be 6 to 9 h.11) Assuming that the absorbed fraction of 5-ASA is completely excreted into the urine as 5-ASA and Ac-5-ASA, we assume that only a small amount of 5-ASA (an estimate of approximately 10%) is absorbed from the gastrointestinal tract when 5-ASA is administered as an enema, whereas the absorbed 5-ASA is extensively acetylated in the liver and the acetylated metabolite Ac-5-ASA is excreted into the urine.

It is generally considered that in the case of SP, the AUC value representing the duration of exposure in the body should be as small as possible to avoid SP-induced side effects. However, the present study found that the AUC value of SP after the administration of the SASP suppository, at about 19 \( \mu \text{M} \cdot \text{h} \), was relatively high. In addition, the AUC value of SASP after the administration of the SASP suppository was nearly equal to that of 5-ASA after the administration of the enema (Tables 1, 2). These findings suggest that the bioavailability of SASP and 5-ASA after administration of respective preparations is nearly equal, but the rate of bioavailability of 5-ASA can be expected to be greater than that of SASP.

In summary, the present study in Japanese volunteers suggested that only about 10% of 5-ASA administered is absorbed and that absorbed 5-ASA is N-acetylated systemically to Ac-5-ASA. It is likely that the 5-ASA enema rather than the SASP suppository is a more effective pharmaceutical preparation for treating patients with ulcerative colitis, although the number of subjects studied was small. Further studies are needed to investigate clinical efficacy in Japanese patients with ulcerative colitis.

REFERENCES