Colon-Specific Prodrugs of 5-Aminosalicylic Acid: Synthesis and *In Vitro/In Vivo* Properties of Acidic Amino Acid Derivatives of 5-Aminosalicylic Acid

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ABSTRACT: 5-Aminosalicyl-L-aspartic acid (5-ASA-Asp) and 5-aminosalicyl-L-glutamic acid (5-ASA-Glu) were synthesized and their properties as colon-specific prodrugs of 5-aminosalicylic acid (5-ASA) were investigated employing rats as test animals. Incubation of 5-ASA-Asp and 5-ASA-Glu with the homogenates of tissue and contents of stomach or small intestine released no 5-ASA, indicating that they were stable in this condition. Incubation of 5-ASA-Asp with the cecal contents released 5-ASA 37%, whereas 5-ASA-Glu released only 8% of the dose in 16 h. Plasma concentration of 5-ASA-Asp after intravenous administration decreased rapidly and became undetectable in 60 min. No 5-ASA was detected in the blood, which indicated 5-ASA-Asp was stable in the plasma. After oral administration of 5-ASA-Asp, concentration of 5-ASA, its metabolite *N*-acetyl-5-ASA, and 5-ASA-Asp in the plasma, feces, and urine was determined. In the plasma, 5-ASA-Asp was not detected and the concentration of 5-ASA or *N*-acetyl-5-ASA was very low. About 33% of the administered dose was recovered as 5-ASA and *N*-acetyl-5-ASA and 43% as 5-ASA-Asp from feces, and 20% as 5-ASA and *N*-acetyl-5-ASA and 1% as 5-ASA-Asp from urine in 24 h. These results suggested that most of 5-ASA-Asp was delivered to the large intestine and about half of the administered dose was activated to liberate 5-ASA. After oral administration of free 5-ASA, fecal recovery was only 7% of the dose in 24 h and more than 80% was recovered from urine. Comparing 5-ASA-Asp and free 5-ASA, the amount of 5-ASA available in the large intestine was much larger, while the amount of 5-ASA in urine, which might be related to the systemic toxicity of 5-ASA, was much lower by the administration of 5-ASA-Asp than free 5-ASA. J Pharm Sci 90:1767–1775, 2001

Keywords: 5-aminosalicyl-L-aspartic acid; 5-aminosalicyl-L-glutamic acid; colon-specific prodrug of 5-aminosalicylic acid; inflammatory bowel disease

INTRODUCTION

Colon-specific delivery of orally administered drugs can be of advantage for the following cases.[1–6] Firstly, for the efficient treatment of diseases that develop at the colonic site, in order to avoid systemic absorption and reduce side effects. Secondly, for those drugs for which absorption through the large intestine is beneficial. For orally administered therapeutic peptides and proteins, the colon could be the preferential site of absorption considering the low level of endogenous enzymes and the long transit time. Thirdly, delayed absorption by colonic targeting may also be useful for the chronotherapy of diseases such as asthma, gastric ulcer, or arthritis, which may have peak symptoms at bedtime. In these cases, the time-plasma level of the active drugs might

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coincide better with the symptoms considering the transit time through the colon.

5-aminosalicylic acid (5-ASA) is an active ingredient of agents used for the long-term maintenance therapy to prevent relapses of Crohn's disease and ulcerative colitis. 5-ASA is absorbed rapidly and extensively in the upper intestine and hardly reaches to the colon.[7] Systemically absorbed 5-ASA is reported to induce nephrotic syndrome.[8] Several prodrugs have been developed aiming at the delivery of 5-ASA to the colon.[9–14]

A colon-specific prodrug should be chemically and biochemically stable and nonabsorbable in the upper intestine so that it can be delivered to the colon in intact form, and the linkage between drug and promoeity should be dissociated to liberate the active drug in the colon.

Amide bonds of various *N*-aromatic acyl-amino acid conjugates are stable in the upper intestine and hydrolyzed when they are incubated with the cecal contents of mammals.[15–17] If a hydrophilic amino acid conjugate of 5-ASA is administered orally, transcellular absorption by way of lipid membrane permeation might be limited in the upper intestine, and a large fraction of the dose might be delivered to the colon in intact form. Once delivered to the colon, 5-ASA might be released from *N*-5-aminosalicyl-amino acid by the microbial degradation in the large intestine, which should be available for the local action. Our previous study showed that about 50% of the orally administered dose of *N*-5-aminosalicylglycine (5-ASA-Gly) was recovered as 5-ASA (*N*-acetyl-ASA) from feces and about 28% as 5-ASA (*N*-acetyl-ASA) and 14% as 5-ASA-Gly from urine. We expect that an acidic amino acid conjugate of 5-ASA, such as 5-aminosalicyl-L-aspartic acid (5-ASA-Asp) or 5-aminosalicyl-L-glutamic acid (5-ASA-Glu), might be more hydrophilic than 5-ASA-Gly, and the transcellular absorption by way of lipid membrane permeation in the upper intestine might be decreased, accordingly. In this report, synthetic methods and *in vitro*/*in vivo* properties of 5-ASA-Asp and 5-ASA-Glu as colon-specific prodrugs of 5-ASA are presented.

MATERIALS AND METHODS

Materials

5-Nitrosalicylic acid (5-NSA), L-aspartic acid dimethyl ester hydrochloride, L-glutamic acid dimethyl ester hydrochloride, *N*,*N*′-dicyclohexylcarbodiimide (DCC), and 10% Pd/C were purchased from Sigma Chemical Co. (St. Louis, MO) and used as received. Solvents for NMR and HPLC were obtained from Merck, Inc. (Damstadt, Germany). All other chemicals were reagent grade, commercially available products. IR spectra were recorded on a Bomem MB 100 FT-IR spectrophotometer (Bomem). ^{1}H-NMR spectra were taken on a Brucker AC-200 spectrometer (Brucker), and the chemical shifts were in ppm downfield from tetramethylsilane. Melting points were taken on a Mel Tem II (Laboratory Devices, Holliston, MA) and were uncorrected. A Parr 4562 pressure reactor (Parr Instrument Company, Moline, IL) was used for catalytic hydrogenation. An Eyela Mazela-Z tissue homogenizer (Tokyo Rikakikai Co., LTD., Tokyo, Japan) was used for homogenization of the gastrointestinal tracts of rats. An Eppendorf Centrifuge 5415C (Eppendorf, Hamburg, Germany) was used for centrifugation. TLCs were performed on Merck Kieselgel 60 F_{254} and RP-8 F_{254s}. Open column chromatography and low-pressure chromatography were performed on Merck silica gel ($70 \approx 230$ and $230 \approx 400$ mesh) and Merck Lichroprep RP-8 size B ($230 \approx 400$ mesh) columns, respectively. The HPLC system consisted of Model 305 and 306 pumps, a 117 variable UV detector, a Model 234 autoinjector, a Model 805 manometric module, and a Model 811C dynamic mixer from Gilson (Middleton, WI).

Calibration of the Compound in Various Biological Specimens

The tissue and contents of the stomach and small intestine and the contents of cecum or colon of a male Sprague-Dawley rat were obtained and homogenized separately. Each sample was diluted with pH 6.8 isotonic phosphate buffer to the concentration of 10 w/v%. To each 100 μL portion of the homogenate was added 10, 50, 100, or 200 μL of the solution of 5-ASA, *N*-acetyl-5-ASA, 5-ASA-Asp, or 5-ASA-Glu (50 μg equivalent of 5-ASA/mL) in pH 6.8 isotonic phosphate buffer, and an appropriate amount of methanol was added to make the final volume of 1 mL. The standard solutions of 5-ASA, *N*-acetyl-5-ASA, 5-ASA-Asp, or 5-ASA-Glu in concentrations of 0.5, 2.5, 5.0, or 10.0 ppm in various biological specimens were obtained by the preceding processes. A calibration curve for 5-ASA, *N*-acetyl-5-ASA, 5-ASA-Asp, or 5-ASA-Glu was constructed from the concentration of the standard solution

versus the peak area on HPLC, which was determined by the following procedure. Standard or blank solution (1 mL) was mixed in a vortex mixer for 2 min, centrifuged at $10{,}000 \times g$ for 5 min, and filtered through a membrane filter (0.45 μm). The filtrate (20 μL) was injected on a Synchropac ODS column (250 × 4.6 mm, 5 μm) and eluted with the mobile phase at a flow rate of 1.5 mL/min and at a pressure of about 2000 psi. The mobile phase consisted of 10% methanol in phosphate buffer (pH 6.0) containing 0.5 mM tetrabutylammonium chloride, which was filtered through a 0.45 μm membrane filter before use. The eluate was monitored at 254 nm by a UV detector measuring the absorption with a sensitivity of AUFS 0.01. The Gilson 712 software was used for the data analysis. The retention time of 5-ASA, *N*-acetyl-5-ASA, 5-ASA-Asp, and 5-ASA-Glu was 280 s, 820 s, 360 s, and 470 s, respectively. Concentration of 5-ASA, *N*-acetyl-5-ASA, 5-ASA-Asp, or 5-ASA-Glu in the sample was calculated from the calibration curve.

Synthesis

5-NSA (5 g, 27.3 mmol) was dissolved in 170 mL of anhydrous ethyl acetate and DCC (6.2 g, 30.0 mmol) was added in portions, with stirring, at 0°C for 1 h. To the reaction mixture, L-aspartic acid dimethyl ester (4.45 g, 27.3 mmol) was added, and the mixture was stirred mechanically for 3 h at 0°C and for 72 h at room temperature. After filtration, the filtrate was evaporated under reduced pressure to remove the solvent. The oily residue was extracted with a saturated solution of $NaHCO_3$. The combined extract was acidified with 3N HCl, extracted with ethyl acetate, dried with anhydrous Na_2SO_4, and the solvent was removed under reduced pressure. The residue was loaded on a silica gel open column and eluted with $CHCl_3$/MeOH (100/1.5), from which 5-nitrosalicyl-L-aspartic acid dimethyl ester was obtained [5.1 g, 58% yield; mp: 149 ≈ 150°C; IR (nujol) ν_{max}(C=O): 1640, 1725, 1749 cm^{-1}; ^1H NMR ($CDCl_3$): 2.9 ≈ 3.2 (2H), 3.7 (s, 3H), 3.8 (s, 3H), 5.0 (m, 1H), 7.0 ≈ 8.8 (m, 3H)]. 5-Nitrosalicyl-L-glutamic acid dimethyl ester was obtained by following the same procedure [mp: 75–77°C; IR (nujol) ν_{max}(C=O): 1650, 1717, 1745 cm^{-1}; ^1H NMR ($CDCl_3$): 2.2 (m, 2H), 2.5 (t, 2H), 3.7 (s, 3H), 3.8 (s, 3H), 4.7 (q, 1H), 7.0 ≈ 8.5 (m, 3H)].

5-Nitrosalicyl-L-aspartic acid dimethyl ester (1 g, 3.95 mmol) and 200 mg of 10% Pd/C in methanol (20 mL) were hydrogenated in a Parr pressure reactor at 50 psi for 1 h. After filtering and removal of methanol, 1 N NaOH (30 mL) was added and reacted for 5 h under nitrogen. On adjustment of pH to 3 ≈ 4, 5-ASA-Asp (0.58 g, 70% yield) was obtained as white precipitates [mp: ≈260°C (decomp); IR (nujol) ν_{max}(C=O): 1640, 1690 cm^{-1}; ^1H NMR (DMSO-d_6): 2.8 (d, 2H), 4.7 (q, 1H), 6.0 ≈ 7.2 (m, 3H)]. 5-ASA-Glu (0.35 g, 38% yield) was obtained by following the same procedure [mp: 230 ≈ 232°C (decomp); IR (nujol) ν_{max}(C=O): 1640, 1731 cm^{-1}; ^1H NMR (DMSO-d_6): 2.1 (m, 2H), 2.3 (t, 2H), 4.4 (m, 1H), 6.6 ≈ 7.5 (m, 3H)].

Apparent Partition Coefficient

To a solution (10 mL) of 5-ASA-Asp or 5-ASA-Glu (100 μg equivalent of 5-ASA/mL) in pH 6.8 isotonic phosphate buffer, 10 mL of chloroform presaturated with pH 6.8 isotonic phosphate buffer was added. The mixture was shaken for 10 h and left for 4 h at 37°C. Concentration of 5-ASA-Asp or 5-ASA-Glu in the aqueous phase was analyzed by HPLC. The apparent partition coefficients were calculated by employing the equation

$$(C_o - C_w)/C_w$$

where C_o and C_w represent the initial and equilibrium concentration of the drug in aqueous phase, respectively.

pH Stability

A solution of 5-ASA-Asp or 5-ASA-Glu (100 μg equivalent of 5-ASA/mL) in pH 1.2 hydrochloric acid buffer or in pH 6.8 phosphate buffer was incubated at 37°C. At a predetermined time interval, a 20 μL portion of the solution was removed, and the concentration of 5-ASA was analyzed by HPLC.

Incubation of 5-ASA-Asp or 5-ASA-Glu With the Homogenate of Tissue and Contents of Stomach or Small Intestine of Rats

A male Sprague-Dawley rat was anesthetized by ether and a midline incision was made. Sections of stomach and small intestine were collected separately, homogenized, and diluted to half concentration with isotonic acetate buffer (pH 4.5) for stomach and with isotonic phosphate buffer (pH 6.8) for small intestine. To each microtube, 0.8 mL of 5-ASA-Asp or 5-ASA-Glu solution in pH 6.8 isotonic phosphate buffer (140 μg equivalent of

5-ASA/0.8 mL) and 0.2 g of the above homogenate were added, mixed, and incubated under nitrogen at 37°C. At appropriate time intervals, a sample was taken out and centrifuged at 5,000 rpm for 3 min. Methanol (0.9 mL) was added to the supernatant (0.1 mL). It was vortexed for 2 min, centrifuged for 5 min at $10{,}000 \times g$, and the amount of 5-ASA in a 20 μL portion of the supernatant was analyzed by HPLC as described previously.

Incubation of 5-ASA-Asp or 5-ASA-Glu With the Cecal and Colonic Contents of Rats

The cecal and colonic segments of the intestine were cut open and their contents were collected separately in a glove box, which was previously displaced by nitrogen. A solution of 5-ASA-Asp or 5-ASA-Glu (140 μg equivalent of 5-ASA/0.9 mL) in pH 6.8 isotonic phosphate buffer (0.9 mL) and the gut contents (0.1 g) were placed in a microtube and incubated at 37°C. At appropriate time intervals, the amount of 5-ASA and *N*-acetyl-5-ASA released in the medium was analyzed by HPLC as described previously.

Plasma Concentration of 5-ASA-Asp, 5-ASA, and *N*-acetyl-5-ASA After Intravenous Administration of 5-ASA-Asp

Male Sprague-Dawley rats weighting $200 \approx 250$ g were anesthetized with ether and cannulated with polyethylene tubing SP 45 (0.96 mm o.d.; Natsum, Japan) through the femoral artery, and were then administered 5-ASA-Asp dissolved in 4.0 mL of physiological saline solution via the femoral vein (10 mg equivalent of 5-ASA/kg). Blood samples were collected from the femoral artery with a heparinized syringe at appropriate time intervals and centrifuged at 5,000 rpm for 3 min. To the 0.1 mL portion of separated plasma, 0.9 mL of methanol was added. The mixture was vortexed for 2 min and centrifuged at $10{,}000 \times g$ for 5 min. The amount of 5-ASA and *N*-acetyl-5-ASA in 20 μL of the supernatant was analyzed by HPLC as described previously.

Plasma Concentration of 5-ASA-Asp, 5-ASA, and *N*-acetyl-5-ASA After Oral Administration of 5-ASA-Asp

Male Sprague-Dawley rats had not been given any food for 24 h, but were given water. They were anesthetized with ether and cannulated with polyethylene tubing SP 45 (0.96 mm o.d.; Natsum, Japan) through the femoral artery. 5-ASA-Asp (50 mg equivalent of 5-ASA/kg), which was dissolved in 1.0 mL of physiological saline solution by adding a minimum amount of 1N-NaOH, was administered by oral zonde. Blood collection and analysis of 5-ASA-Asp, 5-ASA, and *N*-acetyl-5-ASA in the blood were carried out by the procedure described in the previous section.

Concentration of 5-ASA-Asp, 5-ASA, and *N*-acetyl-5-ASA in Feces and Urine After Oral Administration of 5-ASA-Asp

A male Sprague-Dawley rat was placed in a metabolic cage and starved for 24 h prior to use in the experiments, but had free access to water. 5-ASA-Asp (50 mg equivalent of 5-ASA/kg) was orally administered as described previously. The fecal and urinary samples were collected separately at 2 h intervals and stored immediately in a freezer before being analyzed by the following procedure. The fecal or urinary sample was diluted with isotonic phosphate buffer solution (pH 6.8) in 100- or 10-fold, respectively, and centrifuged at 5,000 rpm for 3 min. Methanol (0.9 mL) was added to the supernatant (0.1 mL). The mixture was vortexed for 2 min, centrifuged for 5 min at $10{,}000 \times g$, and the amount of 5-ASA-Asp, 5-ASA, and *N*-acetyl-5-ASA in 20 μL of the supernatant was analyzed by HPLC as described previously.

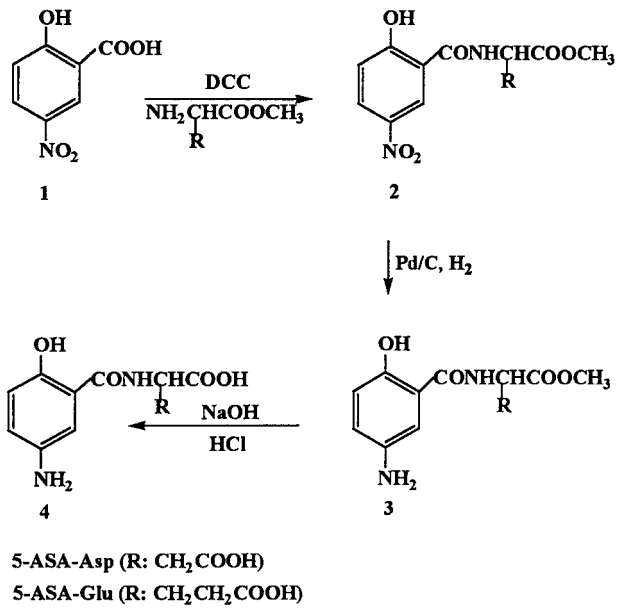
RESULTS AND DISCUSSION

Preparation of 5-ASA-Asp and 5-ASA-Glu

5-ASA-Asp and 5-ASA-Glu were prepared from 5-NSA as shown in Scheme 1, where 5-NSA (1) was directly reacted with dimethyl ester of amino acid in the presence of DCC to produce dimethyl ester of 5-NSA-Asp or 5-NSA-Glu (2). Reduction with 10% Pd/C and subsequent hydrolysis of the ester (3) afforded 5-ASA-Asp or 5-ASA-Glu (4).

Chemical Stability and Apparent Partition Coefficient

Apparent partition coefficient of 5-ASA-Asp and 5-ASA-Glu in chloroform/phosphate buffer (pH 6.8) was 0.03 and 0.04, respectively, at 37°C. Absorption of these compounds by way of trans-



Scheme 1. Synthesis of 5-aminosalicyl-L-aspartic acid and 5-aminosalicyl-L-glutamic acid.

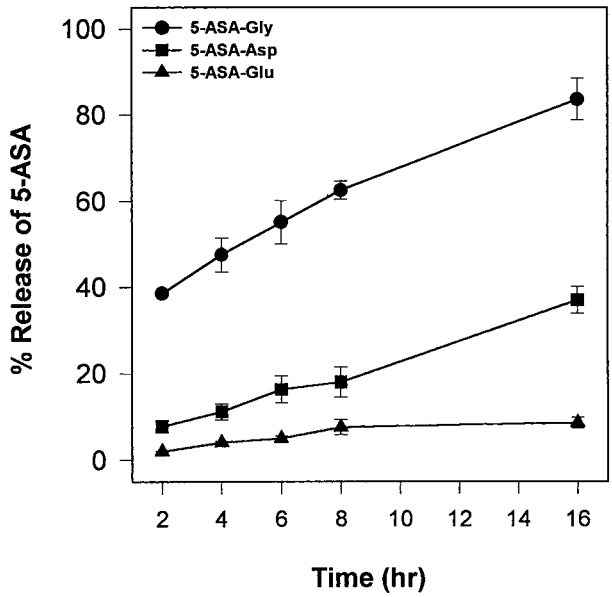


Figure 1. Release profiles of 5-ASA from prodrugs (equivalent to 140 μg of 5-ASA) during incubation with the cecal contents (1.0 mL of 10-fold dilution in pH 6.8 isotonic phosphate buffer) of rats at 37°C. Data are mean ± S.E. ($n = 5$).

cellular passive diffusion might be limited due to the low partition coefficients, unless they are absorbed by paracellular pathways or carrier-mediated transport as in 5-ASA.[18]

5-ASA-Asp and 5-ASA-Glu were incubated at 37°C in pH 1.2 hydrochloric acid buffer or pH 6.8 phosphate buffer solution representing pH of the stomach or small intestine, respectively. No 5-ASA was detected during the 6 h incubation period, which suggested that they might be chemically stable during the transit through the stomach and upper intestine.

Incubation With Homogenates of Various Segments of Gastrointestinal Tract of Rats

5-ASA-Asp and 5-ASA-Glu were incubated with various segments of GI tracts of rats at 37°C, and the release profiles of 5-ASA were determined. The results are shown in Figures 1 and 2. No 5-ASA was detected when 5-ASA-Asp or 5-ASA-Glu was incubated with the homogenate of tissue and contents of stomach or small intestine, suggesting that the prodrug was stable against hydrolysis by peptidases in the upper intestine. When 5-ASA-Asp was incubated with the cecal and colonic contents, the degree of 5-ASA released was about 37 and 9%, respectively in 16 h. Prodrug activation took place most readily in the rat cecum, where the bacterial counts are as high as in the human colon. When 5-ASA-Glu was incubated with the cecal contents, the degree of 5-ASA released was about 8% in 16 h, which was much lower than with 5-ASA-Asp. As shown in Figure 2, the degree of 5-ASA released from 5-ASA-Gly, 5-ASA-Asp, and 5-ASA-Glu upon incubation with the cecal contents was in the order of 5-ASA-Gly > 5-ASA-Asp > 5-ASA-Glu. We believe that steric hindrance imposed by the substituent at α-position inhibited the hydrolysis of the amide bond by the microbes. *N*-acetyl-5-ASA was not detected, which confirms earlier findings that acetylation of the aromatic amino group does not take place by the bacterial systems in the intestine.[7,14,17]

Because the degree of prodrug conversion from incubation of the cecal contents and 5-ASA-Glu was only 8% in 16 h, we did not perform any further studies with 5-ASA-Glu.

Plasma Concentration Profiles After Intravenous Administration of 5-ASA-Asp

5-ASA-Asp was administered intravenously and plasma concentrations of 5-ASA-Asp, 5-ASA, and *N*-acetyl-5-ASA were investigated. The results are shown in Figure 3. Plasma concentration of 5-ASA-Asp decreased rapidly and became undetectable in 60 min. 5-ASA or *N*-acety-5-ASA

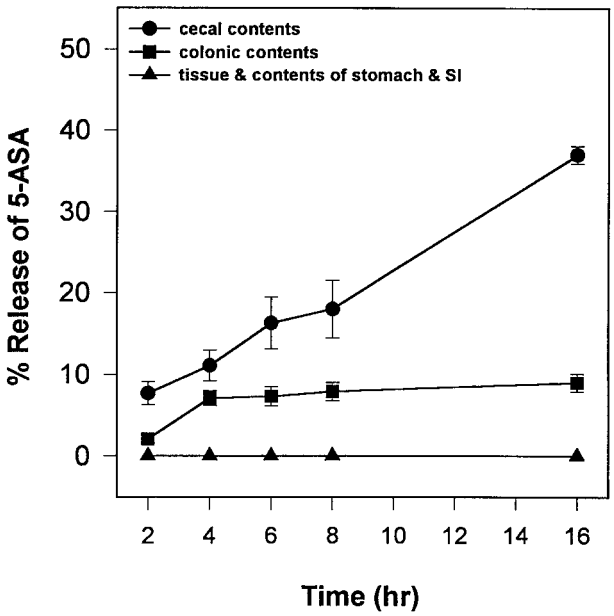


Figure 2. Release profiles of 5-ASA during incubation of 5-ASA-Asp with homogenates of various segments of GI tracts of rats at 37°C. 5-ASA-Asp (equivalent to 140 μg of 5-ASA) in 1.0 mL of 10-fold dilution of : ●, cecal contents; ■, colonic contents; ▲, stomach or small intestinal tissue contents in isotonic phosphate buffer (pH 6.8). Data are mean ± S.E. ($n = 5$).

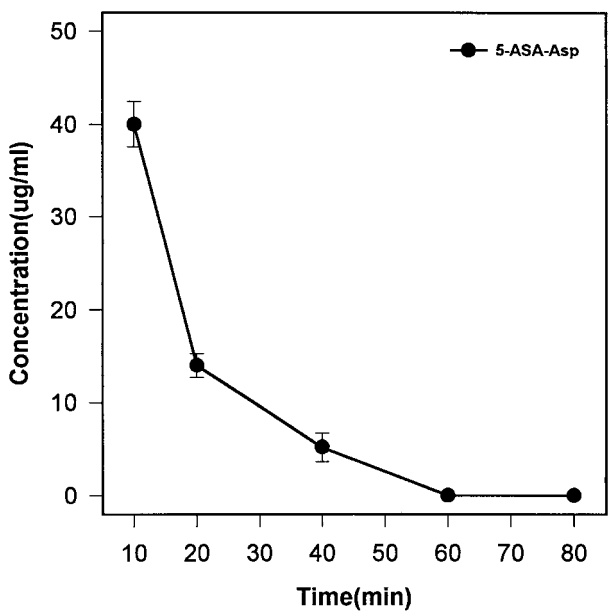


Figure 3. Plasma concentration profiles of 5-ASA-Asp after intravenous administration of 5-ASA-Asp (10 mg equivalent of 5-ASA/kg) via femoral vein of rats. Blood was collected at predetermined intervals from a cannulated femoral artery. 5-ASA and *N*-acetyl-5-ASA were not detected in the plasma. Data are mean ± S.E. ($n = 5$).

was not detected in the blood, implying that conversion of 5-ASA-Asp to 5-ASA did not take place in the blood.

Plasma Concentration Profiles After Oral Administration of 5-ASA-Asp

5-ASA-Asp was administered orally and plasma concentrations of 5-ASA-Asp, 5-ASA, and *N*-acetyl-5-ASA were determined. 5-ASA-Asp was not detected in the plasma, which suggested that absorption of 5-ASA-Asp by way of transcellular passive diffusion is limited due to the low partition coefficient, and paracellular pathways or carrier-mediated transport may not take place. 5-ASA and *N*-acetyl-5-ASA appeared at very low levels during the 24 h experimental period (Figure 4). Considering that 5-ASA-Asp was not converted to 5-ASA in the plasma after intravenous administration (Figure 3), 5-ASA and *N*-acetyl-5-ASA determined in the plasma might be originated from microbially derived 5-ASA from 5-ASA-Asp in the cecum or colon. Thus, 5-ASA-Asp, which is delivered to the large intestine and activated by microbial action, releases 5-ASA. A portion of the released 5-ASA was absorbed in the large intestine and appeared in the plasma. Elimination of 5-ASA and its

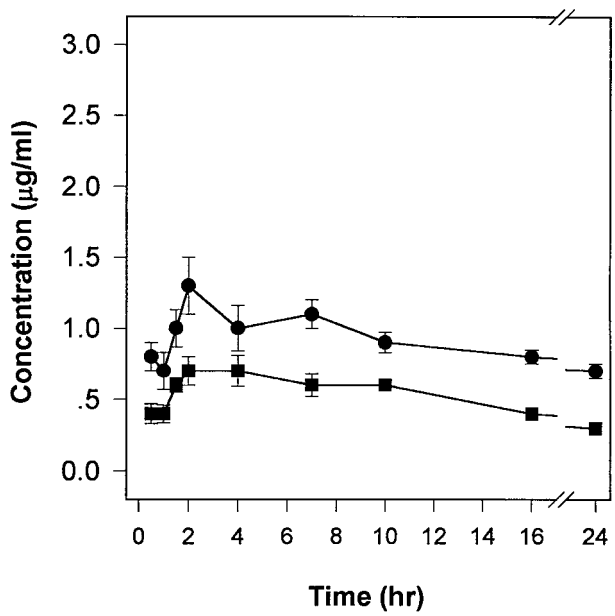


Figure 4. Plasma concentration profiles of 5-ASA (■) and *N*-acetyl-5-ASA (◆) after oral administration of 5-ASA-Asp (50 mg equivalent of 5-ASA/kg) in rats. Blood was collected at predetermined intervals from a cannulated femoral artery. 5-ASA-Asp was not detected in the plasma. Data are mean ± S.E. ($n = 5$).

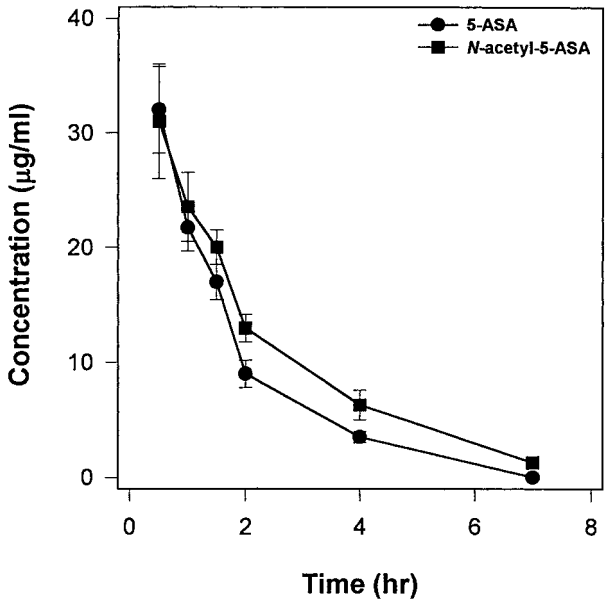


Figure 5. Plasma concentration profile of 5-ASA and *N*-acetyl-5-ASA after oral administration of 5-ASA (50 mg/kg) in rats. Blood was collected at predetermined intervals from a cannulated femoral artery. Data are mean ± S.E. ($n = 5$).

metabolite *N*-acetyl-5-ASA from the plasma was very rapid after oral administration of free 5-ASA (Figure 5). If 5-ASA is gradually released from 5-ASA-Asp and absorbed from the colon, 5-ASA and *N*-acetyl-5-ASA should appear in the plasma at low levels for an extended period of time, as shown in Figure 4.

Recovery of 5-ASA-Asp, 5-ASA, and *N*-acetyl-5-ASA From Feces and Urine After Oral Administration

After oral administration of 5-ASA-Asp, concentration of 5-ASA-Asp, 5-ASA, and *N*-acetyl-5-ASA in feces and urine was determined. The fraction of the dose (%) recovered as 5-ASA, *N*-acetyl-5-ASA, and 5-ASA-Asp from feces was 20.0, 12.5, and 43.0, respectively, and that from urine was 6.5, 13.0, and 1, respectively, in 24 h. These results suggested that most of 5-ASA-Asp was delivered to the large intestine and about one-half of them were activated to liberate 5-ASA. A portion of the liberated 5-ASA was absorbed in the large intestine and eliminated through urine along with its metabolite, *N*-acetyl-5-ASA. Only a small fraction of 5-ASA-Asp was absorbed, which was eliminated rapidly through urine ($\approx 1\%$), rendering the plasma concentration level of 5-ASA-Asp undetectable (Figure 5). Figure 6 compares the results from 5-ASA-Asp, 5-ASA, and sulfasalazine, one of the most commonly prescribed prodrugs of 5-ASA. The fraction of the dose (%) recovered as *N*-acetyl-5-ASA and 5-ASA in feces

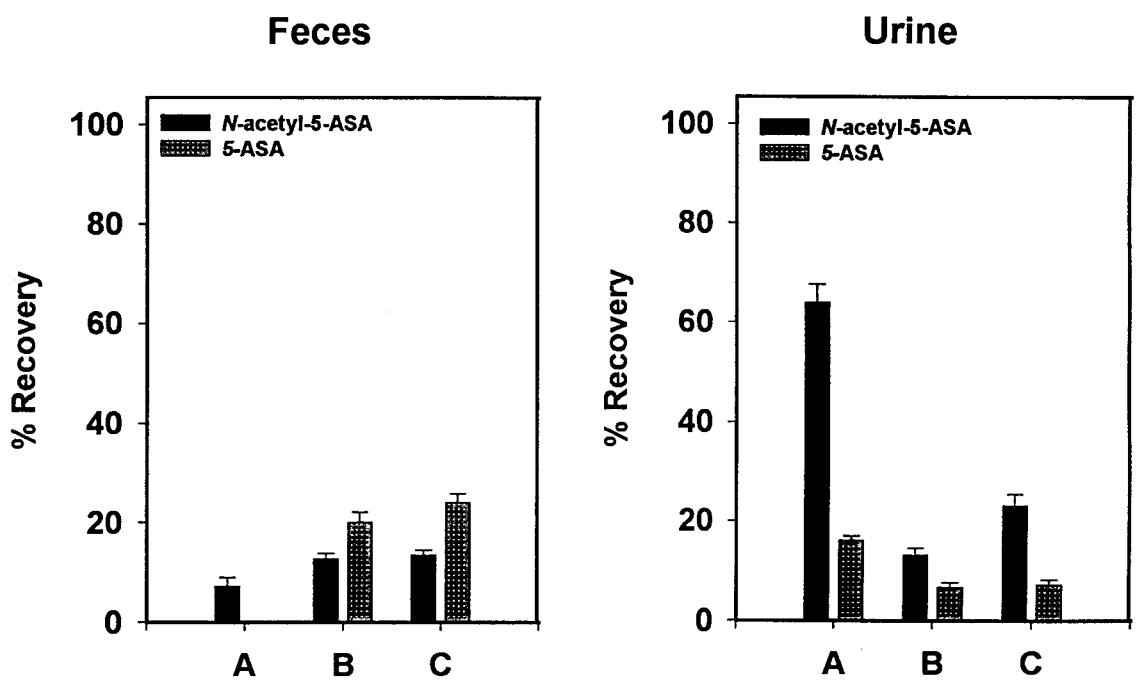


Figure 6. Percent recovery of 5-ASA and *N*-acetyl-5-ASA in feces and urine after oral administration of (A) 5-ASA, (B) 5-ASA-Asp, or (C) sulfasalazine (50 mg equivalent of 5-ASA/kg) in rats. Data are mean ± S.E. ($n = 5$).

was only 7.0 and 0.3, respectively, for 5-ASA[14] (A in Figure 6), 12.5 and 20.0, respectively, for 5-ASA-Asp (B in Figure 6), and 13 and 24, respectively, for sulfasalazine[14] (C in Figure 6). Recovery of 5-ASA-Asp from feces was 43% and that from urine was 1% (not shown in Figure 6).

According to Zhou et al.,[18] part of the absorbed 5-ASA is metabolized to *N*-acetyl-5-ASA, which is secreted into the intestinal lumen predominantly. The fraction of 5-ASA absorbed transcellularly and not metabolized or transported paracellularly enters the general circulation, where it is metabolized in the liver or kidney and finally excreted through urine as an intact drug and metabolite. They reasoned that metabolite levels in the urine represent systemic or hepatic metabolism, and lumenal and fecal metabolite levels represent a measure of intestinal metabolism. Therefore, the fraction of *N*-acetyl-5-ASA in the lumen or feces might be related to therapeutic effect, while that in the urine might be related to side effects.

The fraction of the dose recovered as *N*-acetyl-5-ASA and 5-ASA in feces for 5-ASA-Asp was 32.5%, which is comparable to sulfasalazine (37.3%). The fraction recovered as prodrugs from feces and urine was 43.0 and 1%, respectively. If we compare these results with those from 5-ASA-Gly from our previous study,[14] the degree of prodrug conversion and the fraction of prodrug absorbed (fraction recovered in the urine) decreased by substituting the amino acid from glycine (5-ASA-Gly) with aspartic acid (5-ASA-Asp). Substitution of glycine with aspartic or glutamic acid increased hydrophilicity as well as the steric hindrance at the α-position of the amide bond of the prodrug, which decreased intestinal absorption of the drug by passive diffusion in the upper intestine and the conversion of prodrug by the microbes in the large intestine.

CONCLUSION

Synthesis of 5-ASA-Asp and 5-ASA-Glu was achieved in good yield by a simple synthetic route. Incubation of 5-ASA-Asp and 5-ASA-Glu with the cecal contents of rats released 5-ASA 37 and 8%, respectively, in 16 h, which was much lower than 80% of 5-ASA-Gly in the same conditions. The fraction of the dose recovered as *N*-acetyl-5-ASA and 5-ASA in feces, which might be related to therapeutic effect for 5-ASA-Asp, was 32.5%, which is comparable to sulfasalazine (37.3%), one of the most frequently prescribed prodrugs of 5-ASA. Inertness of a carrier molecule (aspartic acid) might be an advantage of 5-ASA-Asp compared with sulfasalazine, where undesirable side effects originated from the carrier molecule (sulfapyridine) are reported.

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