Synthesis and *In Vitro/In Vivo* Evaluation of 5-Aminosalicyl-Glycine as a Colon-Specific Prodrug of 5-Aminosalicylic Acid

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ABSTRACT: A simple synthetic route for the preparation of amino acid conjugate of 5-aminosalicylic acid (5-ASA) was exploited and prepared 5-aminosalicyl-glycine (5-ASA-Gly) in good yield. *In vitro* and *in vivo* properties of 5-ASA-Gly as a colon-specific prodrug of 5-ASA were investigated using rats as the test animal. Incubation of 5-ASA-Gly with cecal or colonic contents at 37°C released 5-ASA in 65 or 27% of the dose in 8 h, respectively. No 5-ASA was detected from the incubation of 5-ASA-Gly with the homogenates of stomach or small intestine. Plasma concentration of 5-ASA-Gly decreased rapidly after intravenous administration of 5-ASA-Gly, and no 5-ASA was detected in the blood, which indicated 5-ASA-Gly was not degraded in the plasma. After oral administration of 5-ASA-Gly, about 50% of the administered dose was recovered as 5-ASA and *N*-acetyl-ASA and 3% as 5-ASA-Gly from feces and 14% as 5-ASA-Gly and 28% as 5-ASA and *N*-acetyl-ASA from urine in 24 h. These results suggested that a large fraction of 5-ASA-Gly was delivered to the large intestine and activated to liberate 5-ASA. For comparison, total recovery of 5-ASA and *N*-acetyl-5-ASA from feces after oral administration of 5-ASA-Gly was greater than that from sulfasalazine, which is one of the most commonly prescribed prodrugs of 5-ASA. © 2000 Wiley-Liss, Inc. and the American Pharmaceutical Association J Pharm Sci 89:594–602, 2000

Keywords: colon-specific prodrug of 5-aminosalicylic acid; 5-aminosalicylic acid; colon-specific delivery

INTRODUCTION

Delivery of orally administered drugs specifically to the colon is highly desirable for the efficient treatment of diseases developed locally at the colon such as ulcerative colitis, Crohn's disease, constipation, or colorectal cancer. Being delivered specifically to the site of action with limited systemic absorption, only a small dose is needed, which subsequently results in reduced side effects. 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.[1–6] Delayed absorption by colonic targeting may also be useful for the treatment of diseases susceptible to diurnal rhythm such as asthma, gastric ulcer, or arthritis, which may have peak symptoms at bedtime.

Inflammatory bowel diseases (IBD) such as Crohn's disease and ulcerative colitis have not been rare among Europeans and Americans. The exact cause of the IBD is not clearly understood yet, and corticosteroids and salicylates are frequently used therapeutic agents to relieve symptoms of such ailments.[7] 5-ASA is an active ingredient of agents used for the long-term maintenance therapy to prevent relapses of Crohn's

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disease and ulcerative colitis. Unless it is formulated as sustained-release preparations or prodrugs for colon-specific delivery, 5-ASA is not adequate to be used as such because it is absorbed rapidly and extensively through the upper intestine before it reaches to the colon.[8] In addition, systemically absorbed 5-ASA is reported to induce nephrotic syndrome.[9]

Prodrugs aiming at the delivery of 5-ASA to the colon have been introduced that use polymers[10–12] or highly hydrophilic small molecules[13–16] as carriers. Coupling of 5-ASA to the carrier molecule achieved most commonly by means of azo bond as exemplified by sulfasalazine (5-[[4-(2-pyridylsulfamoyl)phenyl]azo]salicylic acid) or olsalazine (disodium 3,3′-azobis(6-hydroxybenzoate)) is based on the fact that azo bonds are readily dissociated by reduction in the large intestine. Glycosides[14] or amide bonds[16] are also applied for attaching 5-ASA to the carrier molecule because these bonds are also dissociated in the large intestine mostly by the microbial action.

To be colon-specific, a prodrug of 5-ASA should be chemically and biochemically stable and nonabsorbable in the upper intestine so that it could be delivered to the colon in intact form. And the linkage between the drug and the promoiety should be dissociated to liberate the active drug in the colon.

N-acyl amide bond derived from an aromatic carboxylic acid and an amino acid is reported to be chemically and biochemically stable in the upper intestine and dissociated in the large intestine by the microbial action.[17] It is also reported that *p*-aminohippuric acid dissociated into *p*-aminobenzoic acid and glycine when it was incubated with rat cecal contents.[18] Glycine or taurine conjugates of bile acids are known to undergo enterohepatic circulation,[19] which implies that the amide bond between the carboxyl group of a bile acid and the amino group of glycine or taurine is microbially degraded in the intestine.

If an amino acid conjugate of 5-ASA is synthesized, the mechanism of absorption might be changed in comparison with 5-ASA and the absorption by paracellular or carrier-mediated mechanisms might be restricted. If the compound is hydrophilic, transcellular absorption by way of lipid membrane permeation might be limited in the upper intestine. Because the amide bond of *N*-aromatic acyl-amino acid conjugate is reported to be stable in the upper intestine,[17] a large fraction of orally administered *N*-5-aminosalicyl-amino acid might be delivered to the colon in intact form. Once delivered to the colon, it might degrade by the microbial enzyme to release 5-ASA and the amino acid. The released 5-ASA in the large intestine should be available for the local action.

In this study, 5-ASA-Gly was designed as a colon-specific prodrug of 5-ASA. A simple synthetic route for the preparation of amino acid conjugate of 5-ASA in good yield is introduced. *In vitro* and *in vivo* properties of 5-ASA-Gly as a colon-specific prodrug of 5-ASA were evaluated.

MATERIALS AND METHODS

Materials

5-Nitrosalicylic acid (5-NSA), glycine methyl 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 are in ppm down field from tetramethylsilane. Melting points were taken on a Mel Tem II (Laboratory Devices, Holliston, MA, USA) and were uncorrected. A Parr 4562 pressure reactor (Parr Instrument Company, Moline, IL, USA) 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. A Hanil Supra K-22 centrifuge (Hanil Instrument, Seoul, Korea) was used for centrifugation. TLCs were performed on Merck Kieselgel 60 F_{254}, and RP-8 F_{254s} (Merck, Damstadt, Germany). Open-column chromatography and low-pressure chromatography were performed on Merck silica gel (70 ~ 230 mesh and 230 ~ 400 mesh), and Merck Lichroprep RP-8 size B (230 ~ 400 mesh) column, respectively. The HPLC system consisted of Model 305, 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, USA).

Calibration of 5-ASA, *N*-acetyl-5-ASA, and 5-ASA-Gly in Various Biologic Specimens

A male Sprague-Dawley rat was anesthetized by diethyl ether, and a midline incision was made

and various segments of the gastrointestinal tract were obtained. The tissue and contents of the stomach and small intestine and the contents of the cecum or colon were homogenized and diluted with isotonic phosphate buffer (pH 6.8) to the concentration of 10 w/v%. To each 100 μL portion of the homogenate, 10, 50, 100, or 200 μL portion of the solution of 5-ASA, 5-ASA-Gly, or *N*-acetyl-5-ASA (50μg equivalent of 5-ASA/mL) in isotonic phosphate buffer (pH 6.8) and an appropriate amount of methanol were added to make the final volume of 1 mL. The standard solutions in concentration of 0.5, 2.5, 5.0, or 10.0μg 5-ASA/mL (or equivalent) were obtained by the preceding processes. A calibration curve for 5-ASA, 5-ASA-Gly, or *N*-acetyl-5-ASA 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 on a vortex mixer for 2 min, centrifuged at 10,000 × *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) (Gilson) 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 m*M* tetrabutylammonium chloride, which was filtered through 0.45 μm membrane filter before use. The eluate was monitored by measuring the absorption at 254 nm at 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, or 5-ASA-Gly was 280 s, 820 s, or 370 s, respectively. Concentration of 5-ASA, 5-ASA-Gly, or *N*-acetyl-5-ASA in the sample was determined from the calibration curve.

Preparation of 5-ASA-Gly

To the solution of 5-nitrosalicylic acid (5 g, 27.3 mmol) in 170 mL of anhydrous ethyl acetate, DCC (6.2 g, 30.0 mmol) was added in portions with stirring at 0°C for 1 h. To the reaction mixture, glycine methyl ester (2.4 g, 27.3 mmol) was added and stirred mechanically for 3 h at 0°C and 72 h at room temperature. After filtration, the filtrate was evaporated under reduced pressure to remove the solvent. The oily residue thus obtained 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 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-glycine methyl ester was obtained (4.4 g, 65% yield). mp: 140 ~ 142°C; IR (nujol) ν_{max}(C=O): 1,635 cm^{-1}, 1,747 cm^{-1}, ^{1}H NMR ($CDCl_3$): 3.8 (s, 3H, $COOCH_3$), 4.9 (d, 2H, $NHCH_2CO$), 7.0 ~ 8.8 (m, 3H, ArH).

5-Nitrosalicyl-glycine methyl ester (1 g, 3.95 mmol) and 200 mg of 10% Pd/C in methanol (20 mL) was placed in a Parr pressure reactor and hydrogenated at 50 psi for 1 h. After filtering and removal of methanol, 1N NaOH (30 mL) was added and reacted for 5 h under nitrogen. On adjustment of pH to 3 ~ 4, 5-ASA-Gly (0.69 g, 83% yield) was obtained as white precipitates. mp: ~297°C (decomp); IR (nujol) ν_{max}(C=O): 1,620 cm^{-1}, 1,648 cm^{-1}; ^{1}H NMR (DMSO-d_6): 3.9 (d, 2H, $NHCH_2CO$), 6.6 ~ 7.0 (m, 3H, ArH).

Apparent Partition Coefficient and pH Stability of 5-ASA-Gly

The apparent partition coefficients were determined as follows. To a solution (10 mL) of 5-ASA (100 (g/mL) or 5-ASA-Gly (100 μg equivalent of 5-ASA/mL) in pH 6.8 isotonic phosphate buffer, 10 mL of chloroform (or *n*-octanol) saturated 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 or 5-ASA-Gly in the aqueous phase was analyzed by HPLC. The apparent partition coefficients were calculated by using 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.

The pH stability was determined by preparing the solution of 5-ASA-Gly (140 (g/mL) in pH 1.2 hydrochloric acid buffer or pH 6.8 phosphate buffer and incubating the solution at 37°C for 6 h. At a predetermined time interval, a 20 μL portion of the solution was removed and analyzed the concentration of 5-ASA by HPLC.

Incubation of 5-ASA-Gly with the Homogenate of Stomach or Small Intestine of Rats

A male Sprague-Dawley rat was anesthetized by diethyl ether and a midline incision was made. Sections of stomach and small intestine were collected separately, homogenized, and the homogenate was diluted to half concentration with isotonic acetate buffer (pH 4.5) for stomach and with isotonic phosphate buffer (pH 6.8) for small intes-

tine. We adopted the pH values for stomach and small intestine as 4.5 and 6.8, respectively, which were the reported pH values normally found in the rat gastrointestinal tract.[20–21] To a 0.2-g portion of each homogenate, 0.8 mL of 5-ASA-Gly solution in pH 6.8 isotonic phosphate buffer (140 μg equivalent of 5-ASA) was added and the mixture was incubated for 6 h at 37°C. At appropriate time intervals, the sample was centrifuged at 5,000 rpm for 3 min. To a 0.1-mL portion of the supernatant, 0.9 mL of methanol was added, vortexed for 2 min, and centrifuged for 5 min at 10,000 × *g*. The concentration of 5-ASA or *N*-acetyl-5-ASA in a 20 μL portion of the supernatant was determined by HPLC as described previously.

Incubation of 5-ASA-Gly 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. To a 0.1-g portion of the gut contents, 0.9 mL of 5-ASA-Gly solution in pH 6.8 isotonic phosphate buffer (140 μg equivalent of 5-ASA) was added and the mixture was incubated at 37°C. At appropriate time intervals, the sample was taken out and centrifuged at 5,000 rpm for 3 min. To a 0.1-mL portion of the supernatant, 0.9 mL of methanol was added, vortexed for 2 min, and centrifuged for 5 min at 10,000 × *g*. The concentration of 5-ASA or *N*-acetyl-5-ASA in a 20 μL portion of the supernatant was determined by HPLC as described previously.

Plasma Concentration Profiles after Intravenous Administration of 5-ASA-Gly

Male Sprague-Dawley rats weighting 200~250 g were anesthetized with diethyl ether and cannulated with polyethylene tubing SP 45 (0.96 mm o.d.; Natsum, Japan) through the femoral artery, and administered 10 mg equivalent of 5-ASA/kg dissolved in 4.0 mL of physiologic saline solution through the femoral vein. 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 the separated plasma, 0.9 mL of methanol was added. It was vortexed for 2 min and centrifuged at 10,000 × *g* for 5 min. The concentration of 5-ASA-Gly, 5-ASA, or *N*-acetyl-5-ASA in a 20-μL portion of the supernatant was analyzed by HPLC as described previously.

Plasma Concentration Profiles after Oral Administration of 5-ASA or 5-ASA-Gly

Male Sprague-Dawley rats had been starved for 24 h except for water. They were anesthetized with diethyl ether and cannulated with polyethylene tubing SP 45 (0.96 mm o.d.; Natsum, Japan) through the femoral artery. 5-ASA or 5-ASA-Gly (50 mg equivalent of 5-ASA/kg), which was dissolved in 1.0 mL of physiologic saline solution by adding minimum amount of 1N-NaOH, was administered by an intragastric probe. The same protocol was carried out for the sample collection and analysis of 5-ASA-Gly, 5-ASA, and *N*-acetyl-5-ASA in the blood as described previously.

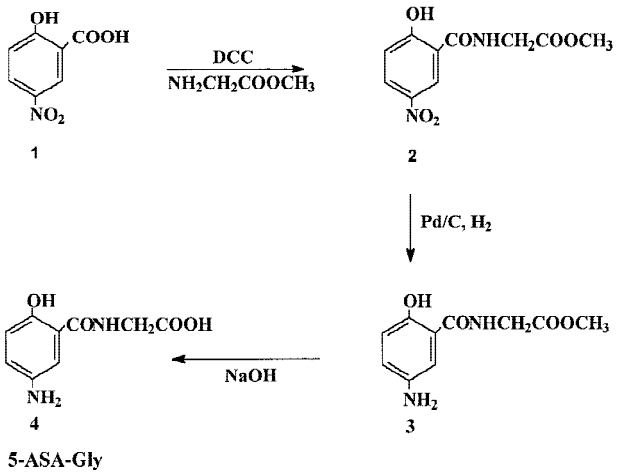
Release of 5-ASA and *N*-acetyl-5-ASA in Feces or Urine after Oral Administration of 5-ASA, 5-ASA-Gly, or Sulfasalazine

Male Sprague-Dawley rats were placed in a metabolic cage and starved for 24 h before use for the experiments but had free access to water. 5-ASA, 5-ASA-Gly, or sulfasalazine (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) to 100-fold or 10-fold, respectively. The sample was vortexed and centrifuged at 5,000 rpm for 3 min. To a 0.1-mL portion of the supernatant, 0.9 mL of methanol was added, vortexed for 2 min, and centrifuged for 5 min at 10,000 × *g*. The concentration of 5-ASA, *N*-acetyl-5-ASA, or 5-ASA-Gly in a 20 μL portion of the supernatant was determined by HPLC as described previously.

RESULTS AND DISCUSSION

Preparation of 5-ASA-Gly

Preparation of 5-ASA-Gly was achieved as shown in Scheme 1: (i) 5-NSA was reacted with glycine methyl ester in the presence of DCC to produce 5-NSA-Gly methyl ester. (ii) Reduction of nitro group with 10% Pd/C gave 5-aminosalicyl-glycine methyl ester (iii), and hydrolysis of the ester afforded 5-ASA-Gly (iv). The overall yield was high,



Scheme 1. Synthesis of 5-aminosalicyl-glycine from 5-nitrosalicylic acid.

and the reaction processes were relatively simple compared with the method using 5-ASA as a starting material.[15]

Apparent Partition Coefficient and pH Stability of 5-ASA and 5-ASA-Gly

Apparent partition coefficient of 5-ASA, which was determined at 37°C using chloroform/ phosphate buffer (pH 6.8) or *n*-octanol/phosphate buffer (pH 6.8), was 0.03 or 0.04, respectively, and that of 5-ASA-Gly was 0.18 or 0.24, respectively. Because 5-ASA possesses a low partition coefficient, it is absorbed in the upper intestine by way of paracellular pathways or carrier-mediated transport rather than transcellular passive diffusion.[25] In the case of 5-ASA-Gly, absorption by transcellular passive diffusion might be limited because of the low partition coefficient, which may result in limited overall absorption in the upper intestine, unless it is absorbed by the same mechanism as 5-ASA.

Chemical stability of 5-ASA-Gly was determined at 37°C in pH 1.2 hydrochloric acid buffer or pH 6.8 phosphate buffer solution. No 5-ASA was detected during the 6 h of the incubation period, which indicated that 5-ASA-Gly might be chemically stable during the transit through the gastrointestinal tract.

Release Profiles of 5-ASA after Incubation of 5-ASA-Gly with Homogenates of Various Segments of Gastrointestinal Tract of Rats

To investigate the hydrolysis of 5-ASA-Gly by the peptidases, which are known to be highly distributed in the mucosal membranes of the small intestine, i*n vitro* experiments to determine the release of 5-ASA after incubation of 5-ASA-Gly with the homogenate of stomach or small intestine (tissue and contents) of rats were carried out at 37°C. Incubation of 5-ASA-Gly with the cecal and colonic contents of rats was also carried out at 37°C, and the results are shown in Figure 1. On incubation of 5-ASA-Gly with the homogenate of stomach or small intestine at 37°C, 5-ASA was not detected, which indicated that the prodrug might be stable against peptidases in the upper intestine. When 5-ASA-Gly was incubated with the cecal contents, about 40% of the dose was released as 5-ASA in 2 h and about 65% in 8 h. With the colonic contents, the fraction of the dose released as 5-ASA was only 27% in 8 h, which indicated that prodrug activation took place most readily in the rat cecum, where the bacterial counts are high as in the human colon. *N*-acetyl-5-ASA, a major metabolite of 5-ASA, was not detected, which confirms the early findings that colonocytic acetylation is responsible for the acetylation of the aromatic amino group rather than bacterial system in the intestine.[8,17]

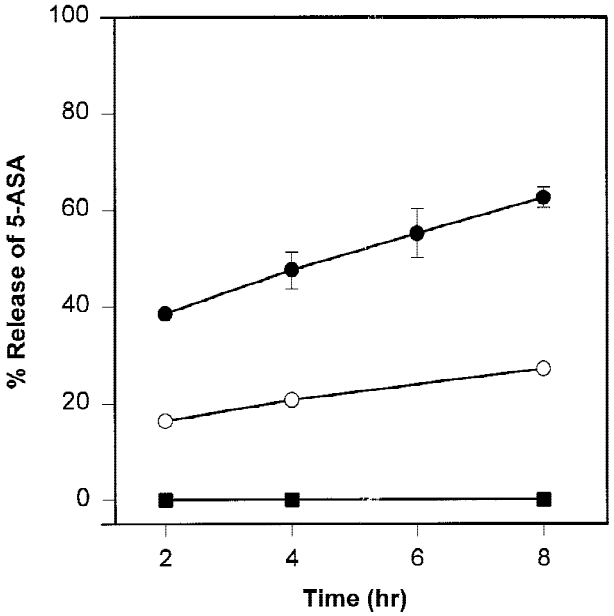


Figure 1. Release profiles of 5-ASA during incubation of 5-ASA-Gly with homogenates of various segments of the gastrointestinal tract of rats at 37°C. 5-ASA-Gly (equiv. to 140μg of 5-ASA) in 1.0 mL of tenfold dilution of (●) cecal contents, (○) colonic contents, (■) stomach and small intestinal tissue/contents in isotonic phosphate buffer (pH 6.8). Data are mean ± SE (n = 5).

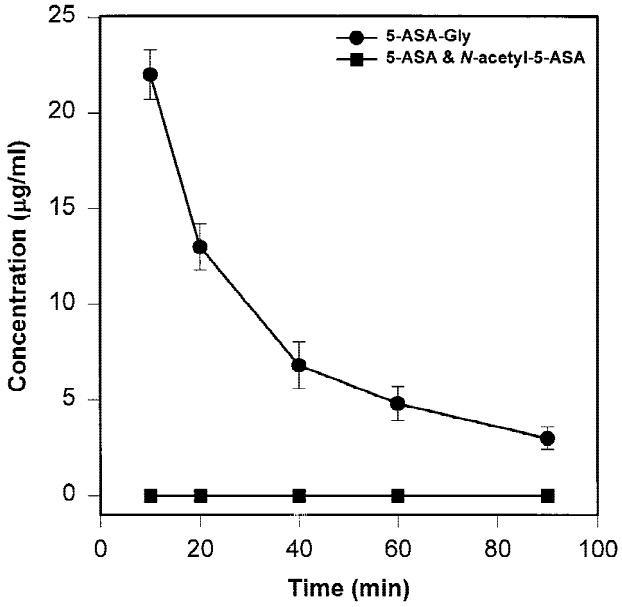


Figure 2. Plasma concentration profiles of 5-ASA-Gly, 5-ASA, and *N*-acetyl-5-ASA after intravenous administration of 5-ASA-Gly (10 mg eqv. of 5-ASA/kg) through the femoral vein of rats. Blood was collected at predetermined interval from a cannulated femoral artery. Data are mean ± SE ($n = 5$).

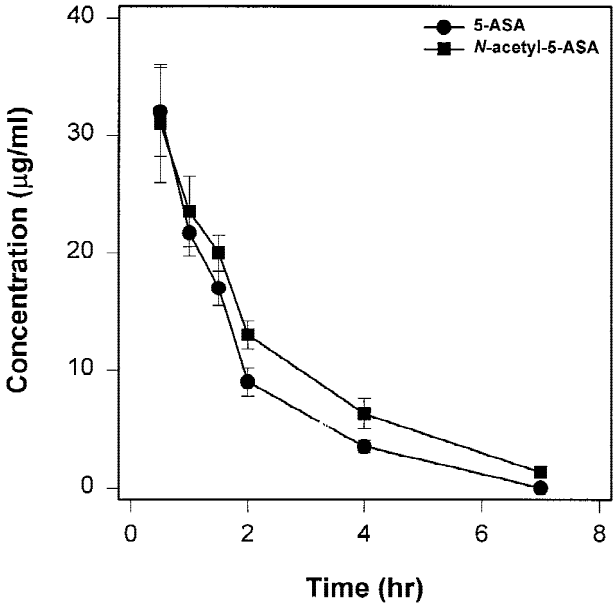


Figure 3. Plasma concentration profile of 5-ASA and *N*-acetyl-5-ASA after oral administration of 5-ASA (50 mg/kg) in rats. Blood was collected at predetermined interval from a cannulated femoral artery. Data are mean ± SE ($n = 5$).

Plasma Concentration Profiles after Intravenous Administration of 5-ASA-Gly

After intravenous administration of 5-ASA-Gly, plasma concentration of 5-ASA-Gly, 5-ASA, or *N*-acetyl-5-ASA was determined by HPLC and the results are shown in Figure 2. Plasma concentration of 5-ASA-Gly decreased rapidly and 5-ASA or *N*-acetyl-5-ASA was not detected, indicating that 5-ASA-Gly was not degraded in the blood and eliminated rapidly.

Plasma Concentration Profiles after Oral Administration of 5-ASA or 5-ASA-Gly

5-ASA or 5-ASA-Gly was administered orally (50 mg equivalent of 5-ASA/kg) and plasma concentration profiles of 5-ASA and *N*-acetyl-5-ASA were determined and compared. Absorption of 5-ASA took place rapidly ($t_{max} \leq 0.5$ h), showing the t_{max} is shorter than the reported values of 1 h.[22] Plasma concentration of *N*-acetyl-5-ASA was higher than that of 5-ASA and elimination was nearly complete in 7 h (Fig. 3).

After oral administration of 5-ASA-Gly, plasma concentration of 5-ASA-Gly, 5-ASA, or *N*-acetyl-5-ASA was determined (Fig. 4). The level of 5-ASA-Gly in the plasma was extremely low throughout the whole experimental period (24 h), which might indicate the limited oral absorption and the facile elimination of 5-ASA-Gly from the plasma as illustrated in Figure 2. The plasma concentration profile of 5-ASA or *N*-Ac-5-ASA showed t_{max} around 2 h, which seemed shorter than usually expected. It is reported that the transit time through the upper intestine of the rat is about the same or slightly shorter than that of the man.[23] The transit time of a tablet (7 mm in diameter) to the large intestine taken with a regular meal is reported to be 1.5 h in man.[24] It might be shortened if the drug were administered as a solution form in fasting state as we did in this experiment. In this experimental condition, the transit time through the upper intestine might have been shortened. This might explain the short t_{max} value for 5-ASA as shown in Figure 3. Shortened transit time through the upper intestine and the facile hydrolysis in the cecum might be responsible for the early detection of 5-ASA and *N*-acetyl-5-ASA in the plasma or the short t_{max} values observed after oral administration of 5-ASA-Gly. The levels of 5-ASA and *N*-acetyl-5-ASA in the plasma were very low for the whole experimental period (24 h), which differs greatly from the case in which the equivalent amount of

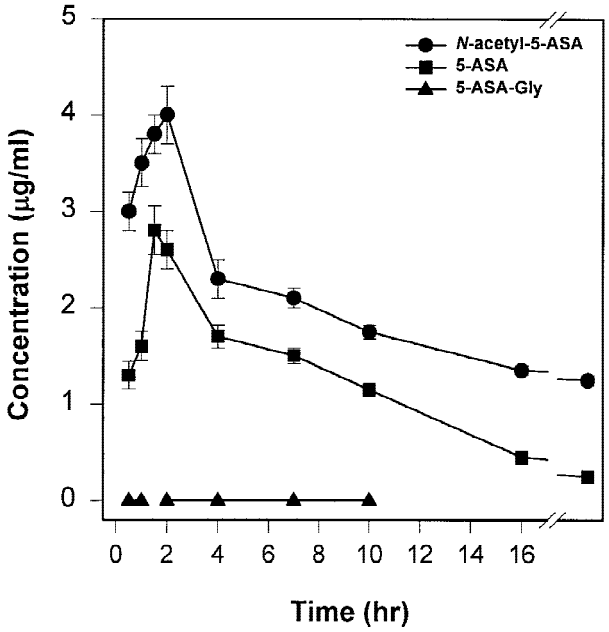


Figure 4. Plasma concentration profiles of 5-ASA-Gly, 5-ASA, and *N*-acetyl-5-ASA after oral administration of 5-ASA-Gly (50 mg eqv. of 5-ASA/kg) in rats. Blood was collected at predetermined interval from a cannulated femoral artery. Data are mean ± SE ($n = 5$).

free 5-ASA was administered orally (Fig. 3). Because 5-ASA-Gly was not degraded to release 5-ASA in the plasma (Fig. 2), or it did not released 5-ASA on incubation with the upper intestinal constituents (Fig. 1), 5-ASA or *N*-acetyl-5-ASA appearing in the plasma might originate from the one absorbed in the large intestine. Activated by the microbial enzyme in the large intestine, 5-ASA-Gly releases 5-ASA at a controlled rate, and a portion of the released 5-ASA might be absorbed in the large intestine and appears in the plasma for an extended period of time.

Recovery of 5-ASA and *N*-acetyl-5-ASA in the Feces and Urine after Oral Administration of 5-ASA-Gly, 5-ASA, or Sulfasalazine

Concentrations of 5-ASA and *N*-acetyl-5-ASA in the feces and urine 24 h after oral administration of 5-ASA-Gly, 5-ASA, or sulfasalazine were determined and the results are shown in Figure 5. Recovery (% of the dose) of 5-ASA from the feces for 5-ASA-Gly, 5-ASA, or sulfasalazine was 31.0, 0.3, or 24.0, respectively. Recovery (% of the dose) of *N*-acetyl-5-ASA from feces for 5-ASA-Gly, 5-ASA, or sulfasalazine was 20.8, 7.0, or 13.3, respectively. From urine, recovery (% of the dose) of 5-ASA for 5-ASA-Gly, 5-ASA, or sulfasalazine was 6.5, 16.0, or 7.0, respectively, and that of *N*-acetyl-5-ASA for 5-ASA-Gly, 5-ASA, or sulfasalazine was 22.0, 64.0, or 23.0, respectively. Recovery (% of the dose) of 5-ASA-Gly from feces or urine was 3.5 or 14.0 (not shown in Fig. 5), respectively.

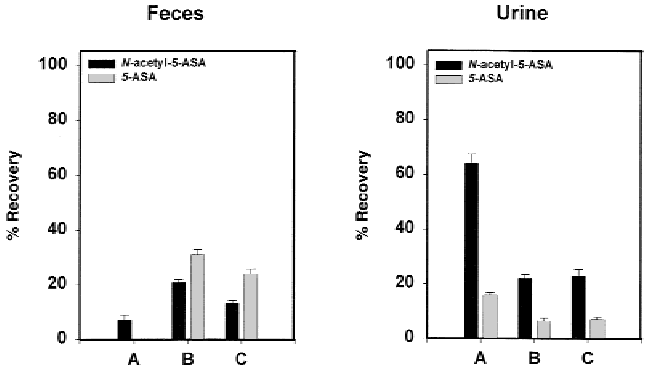


Figure 5. Percent recovery of 5-ASA and *N*-acetyl-5-ASA in feces and urine after oral administration of (A) 5-ASA, (B) 5-ASA-Gly, and (C) sulfasalazine (50 mg eqv. of 5-ASA/kg) in rats. Data are mean ± SE ($n = 5$).

Recently, Zhou et al.[25] reported that a fraction of 5-ASA absorbed in the intestinal cell 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 luminal 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, whereas that in the urine might be relate to side effects.

In summary, only about 7% of the dose was recovered as *N*-acetyl-5-ASA from the feces and about 80% of the dose was recovered as 5-ASA and *N*-acetyl-5-ASA from the urine in 24 h with free 5-ASA. With 5-ASA-Gly, about 21% of the dose was recovered as *N*-acetyl-5-ASA from the feces and about 28% of the dose was recovered as 5-ASA and *N*-acetyl-5-ASA from the urine in 24 h. With sulfasalazine, 13% of the dose was recovered as *N*-acetyl-5-ASA from the feces and 30% of the dose was recovered as 5-ASA and *N*-acetyl-5-ASA from the urine in 24 h.

Results from our *in vivo* studies suggested that a large fraction of 5-ASA-Gly was delivered to the large intestine and activated to liberate 5-ASA (~52%) after oral administration of 5-ASA-Gly. A portion of 5-ASA was absorbed to appear in the plasma (Fig. 4) and eliminated through the urine as an intact drug and metabolite (~28%). Only a small fraction of 5-ASA-Gly might have been absorbed in the upper intestine, which was eliminated rapidly through the urine (~14%), rendering the plasma concentration level below the detection limit (Fig. 4).

CONCLUSION

Preparation of 5-ASA-Gly was achieved in good yield by a simple synthetic route. *In vitro* and *in vivo* experiments to evaluate 5-ASA-Gly as a colon-specific prodrug of 5-ASA were carried out. A substantial amount of 5-ASA was released when 5-ASA-Gly was incubated with cecal or colonic contents but not with upper intestinal tissue homogenate or contents. After intravenous administration, 5-ASA-Gly was not degraded in the plasma and was eliminated rapidly from the plasma. After oral administration, concentration of 5-ASA-Gly in the plasma was less than the limit of detection (0.2 μg/mL), and only a small fraction of 5-ASA-Gly was absorbed and excreted in the urine. Total recovery (%) from the feces, which includes unabsorbed drug and intestinal metabolite, as well as the recovery (%) of *N*-acetyl-5-ASA from the feces, was greater in 5-ASA-Gly than sulfasalazine. Total recovery (%) from the urine was slightly greater in sulfasalazine than 5-ASA-Gly. Inertness of a carrier molecule (glycine) might be an advantage of 5-ASA-Gly compared with sulfasalazine, in which undesirable side effects originating from the carrier molecule (sulfapyridine) are reported. These results suggested that 5-ASA-Gly is a promising colon-specific prodrug of 5-ASA.

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