INTESTINAL METABOLISM AND TRANSPORT OF 5-AMINOSALICYLATE


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ABSTRACT:

The purpose of this study was to determine the characteristics of intestinal absorption and metabolism of 5-aminosalicylic acid (5ASA). Regional perfusions of 5ASA in the anesthetized rat resulted in the appearance of N-acetyl-5-aminosalicylic acid in the intestinal lumen. Lumenal metabolite appearance was proportional to 5ASA permeability, which was 5-fold higher in the jejunum than in the ileum. Intestinal elimination significantly decreases 5ASA absorption at low lumenal drug concentrations and this process is saturated at high drug concentrations. Metabolite levels in intestinal tissue were higher than plasma levels at low perfusion drug concentrations, whereas the reverse was observed at high concentrations. Transport and metabolism of 5ASA was studied in Caco-2 monolayers. At low drug concentrations, 5ASA was preferentially transported in the basolateral (BL) to apical (AP) direction. With 5ASA incubation in either the AP or BL chamber, the N-acetyl metabolite appeared only in the AP compartment. Transport of N-acetyl-5-aminosalicylic acid was also exclusively observed in the BL to AP direction. Clinical data indicate that anti-inflammatory response to oral 5ASA correlates with the amount of 5ASA delivered to the intestinal tissue. This study shows that at lumenal levels below 200 µg/ml (concentrations that are typically achieved by controlled release dosage forms), intestinal secretion of 5ASA accounts for more than 50% of the total elimination and can significantly affect tissue levels and, therefore, may be an important factor in determining the response to 5ASA therapy.

Mammalian intestine has been found to eliminate drugs by oxidative phase 1 and conjugative phase 2 metabolism (de Waziers et al., 1990; Ilett et al., 1990; Krishna and Klotz, 1994). Coupled with intestinal secretion of drug and metabolites, these processes may impact on drug plasma levels by influencing absorption variability (Wacher et al., 1996). These pathways also provide the potential for drug and nutrient interactions to effect drug absorption (Hui et al., 1994). As an important metabolic pathway in the elimination of carcinogenic amines, N-acetylation of arylamines occurs in a number of body organs (Hickman and Sim, 1991). Although primarily studied in the liver, N-acetyltransferase (NAT) has been shown to be distributed evenly along the intestinal tract of hamsters (Ware and Svensson, 1996), mice (Ware and Svensson, 1996), rats (Ware and Svensson, 1996), rabbits (Ilett et al., 1991), and humans (Prueksaritanont et al., 1996; Hickman et al., 1998). Intestinal secretion of N-acetylated drug metabolites has also been reported to play an important compensatory role in the renal excretion of procainamide (Arimori and Nakano, 1988). 5-aminosalicylic acid (5ASA)1, a drug used to treat inflammatory bowel disease, is metabolized by N-acetylation. Efficacy of 5ASA correlates with tissue delivery and, therefore, factors such as intestinal metabolism and elimination that affect tissue delivery may be important in determining drug efficacy. From previous work on intestinal and systemic N-acetylation of 5ASA, there appear to be some open questions on intestinal metabolic capacity and the fate of the intestinal metabolite (Pieniaszek and Bates, 1979; Myres et al., 1987; Bondesen, 1997) in relation to systemic 5ASA elimination. In this paper, the mechanisms and significance of intestinal elimination of 5ASA are explored.

Rat perfusion studies were carried out to examine the appearance of metabolite in the intestinal lumen and to assess the intestinal permeability of 5ASA as a function of intestinal region and perfusion drug concentration. Caco-2 monolayers were studied to determine the role of drug and metabolite secretion in intestinal elimination of 5ASA. Results indicate that the paracellular pathway in the relatively leaky upper small intestine provides for complete absorption of this drug at high solution concentrations. Compared with jejunum, the ileum is less permeable and less high solution concentrations. Compared with jejunum, the ileum is less permeable and less.

Materials and Methods

Chemicals. 5ASA and acetic acid were obtained from Sigma Chemical Co. (St. Louis, MO) and propionic anhydride was purchased from Aldrich Chemical Co. (Milwaukee, WI). 2-(N-morpholino)ethanesulfonic acid (MES), citric...
acids, sodium hydroxide, monobasic potassium phosphate, hydrochloric acid, HPLC grade methyl alcohol, and HPLC grade acetonitrile were purchased from Fisher Scientific Company (Pittsburgh, PA). N-acetyl-5-aminosalicylic acid (N-acetyl 5ASA) was synthesized by reacting 5ASA with acetic anhydride under catalysis of triethylamine in ethyl ester. The N-acetyl 5ASA product was extracted with ethyl ether and recrystallized. Its structure was verified by NMR and its purity was tested using both NMR and HPLC with fluorescence detection (data not shown).

Procedures for Single Pass Perfusion Experiments. Male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA) weighing between 250 and 300 g were fasted for 14 to 17 h before surgery. Rats were anesthetized with 87 mg/kg ketamine and 13 mg/kg xylazine and the small intestine was surgically exposed. Two incisions were made at the proximal and distal end of a 12-cm long segment of jejunum for implantation of inlet and outlet tubing into the lumen for single pass perfusion. A 10-cm length of ileum was similarly prepared in separate animals. Isotonic solutions of different concentration all contained 10 mM MES buffer with 5 mM KCl and 0.1% sodium hydroxide, monobasic potassium phosphate, hydrochloric acid, nonessential amino acids, 1% sodium pyruvate, and 1% penicillin-streptomycin. Cells were passed continuously in petri dishes every 4 days and used between passage number 50 and 60. The Caco-2 monolayers used in transport and metabolism studies were grown on 6-well clear polyester transwells (24 mm insert diameter, 3.0 μm pore size, 4.7 cm² growth area; Corning Costar, Cambridge, MA) seeded with 2 to 4 × 10⁶ cells. After seeding, the cells were stored in a 37°C incubator under 95% CO₂ and 5% O₂ for 21 days for the cells to fully differentiate into confluent enterocyte-like monolayers. Before initiation of transport experiments, monolayer integrity was verified via transmembrane resistance measurements and both sides of the Caco-2 monolayers were washed twice with PBS buffer.

Transport Measurements in Caco-2 Cells. 5ASA and N-acetyl 5ASA flux across Caco-2 monolayers was measured with drug solutions applied in either apical (AP) or BL chambers in the absence of plasma. The pH in the AP chamber was maintained at 6.4 with MES buffer containing 145 mM NaCl, 3 mM KCl, 1 mM NaH₂PO₄, 1 mM CaCl₂, 0.5 mM MgCl₂, 5 mM d-glucose, and 5 mM MES; the pH in the BL chamber was maintained at 7.4 with HEPES buffer (of similar solution composition). After drug application, 50 μl of medium was sampled from both AP and BL chambers at 75 and 175 min.

HPLC Assays. The quantification of 5ASA and N-acetyl 5ASA in intestinal tissue, plasma, and intestinal perfusate was carried out by reversed phase HPLC with fluorescence detection. The HPLC system was composed of a Waters 501 HPLC pump, Waters 770 autosampler, LiChrosorb RP-C₁₈ column (250 × 4.6 mm), ABS980 fluorescence detector (317 nm excitation, 417 nm emission cut off), Shimadzu CR 501 chromatopac integrator, and Anspec linear strip recorder. Perfusate samples were directly injected onto the HPLC and the tissue and plasma samples were derivatized with propionic anhydride. Briefly, 100 μl of plasma or tissue homogenates was derivatized with a 100 μl mixture of ethyl acetate solution of propionic anhydride (66%) and triethylamine (34%). The reaction mixture was vortexed and the reaction was allowed to run to completion over 30 min. After derivatization, 50 μl 10% HCl was added to the reacted mixture to remove the triethylamine from the organic phase and 100 μl ethyl acetate was added to facilitate complete extraction of the N-alkyl 5ASA derivatives into the organic solvent. The phase separation was accelerated by centrifugation at 10,000 rpm for 3 min and 50 μl of the supernatant N-alkyl 5ASA derivatives ethyl acetate solution was transferred into a 1-ml
Ethyl acetate was evaporated under vacuum and the residual \( N \)-alkyl 5ASA derivatives were dissolved in 0.7 ml HPLC mobile phase for direct injection onto the HPLC column. The separation of 5-ASA, \( N \)-acetyl 5ASA, and \( N \)-propionyl 5ASA was completed within 4 min using eluent composed of water/200 mM citric buffer/acetonitrile/methanol (65: 5: 15:15) at a flow rate of 1.6 ml/min. Assay linearity was observed from 1 \( \mu \)g/ml to 7.5 \( \mu \)g/ml \( (r = 0.998) \). The concentration response range was linear from 1 \( \mu \)g/ml to 0.75 \( \mu \)g/ml \( (r = 0.998) \) for 5ASA direct analysis and 0.3 to 75 \( \mu \)g/ml and 0.2 to 75 \( \mu \)g/ml for 5ASA and \( N \)-acetyl 5ASA, respectively, after derivatization.

**Data Analysis and Presentation.** The data obtained were analyzed by one way ANOVA using Sigma Statistic in Sigma Plot software with the significance preset at 95%. Drug loss and lumenal appearance data are presented as a percentage of initial perfusion concentration. Metabolite appearance and levels are measured concentrations. Data is presented as mean values of at least four samples with variability presented as S.D.

**Results**

**Intestinal Perfusion Studies.** The permeability of 5ASA at 7.5 \( \mu \)g/ml in rat jejunum and ileum was measured to be 6.17 \( \pm \) 1.82 \( \times \) \( 10^{-6} \) cm/s and 0.04 \( \pm \) 1.36 \( \times \) \( 10^{-6} \) cm/s, respectively. Metabolite levels in the jejunal and ileal perfusate were 1.04 \( \pm \) 0.02 and 0.17 \( \pm \) 0.05

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**Images:**

1. **Fig. 2.** Steady-state concentration of \( N \)-acetyl 5ASA in the jejunal lumen from jejunal perfusions of 5ASA normalized by the length of the perfused segment.

2. **Fig. 3.** \( N \)-acetyl 5ASA concentration in jejunal tissue versus plasma \( N \)-acetyl 5ASA concentration from steady-state jejunal perfusions at various 5ASA concentrations.

3. **Fig. 4.** Percentage of 5ASA donor chamber concentration appearing in receiver chamber across Caco-2 monolayers as a function of initial 5ASA donor chamber concentration.
At 5 mg/ml, the metabolite generated cannot be separated from 5ASA due to the high concentration of the parent drug.

Jejunal 5ASA metabolism significantly decreased drug absorption at low 5ASA concentration (0.075 mg/ml) but had no significant impact on absorption at high 5ASA concentration (7.5 mg/ml; Fig. 1). Jejunal appearance of N-acetyl 5ASA showed a trend toward saturation with increasing 5ASA perfusate concentration. (Fig. 2). The levels of metabolite in intestinal tissue and plasma from steady-state intestinal perfusion are presented in Fig. 3. The tissue metabolite levels are higher than plasma metabolite levels at low 5ASA concentrations, whereas the plasma metabolite levels are higher than tissue metabolite levels at high perfusion 5ASA concentrations.

**Caco-2 Studies.** Transport of 5ASA across Caco-2 monolayers was studied with drug incubation in either the AP or BL chamber utilizing

![Graph showing concentration of N-acetyl 5ASA appearing in the AP chamber across Caco-2 monolayers as a function of initial donor-chamber 5ASA concentration.](image1)

**Fig. 5.** Concentration of N-acetyl 5ASA appearing in the AP chamber across Caco-2 monolayers as a function of initial donor-chamber 5ASA concentration.

![Graph showing concentration of N-acetyl 5ASA appearing in the AP chamber across Caco-2 monolayers at 75 and 155 min as a function of initial donor-chamber N-acetyl 5ASA concentration.](image2)

**Fig. 6.** Concentration of N-acetyl 5ASA appearing in the AP chamber across Caco-2 monolayers at 75 and 155 min as a function of initial donor-chamber N-acetyl 5ASA concentration.
AP and BL of equal 2-ml volumes. At low incubation concentrations, BL to AP flux was higher than AP to BL, whereas at higher concentrations, flux was independent of direction with the percentage of transport leveling off at approximately 2% (Fig. 4). Regardless of which chamber was the site of initial drug incubation, N-acetyl metabolite was only observed in the AP chamber. Higher metabolite levels were generated when 5ASA of the same concentration was incubated in the AP chamber as compared with the BL chamber (Fig. 5). Apical incubations with N-acetyl 5ASA showed no transport of the metabolite whereas BL incubations resulted in minimal N-acetyl 5ASA appearance in the AP compartment (Fig. 6). Interestingly, very low levels of 5ASA were also observed in both AP and BL chambers from BL incubations with N-acetyl 5ASA (Fig. 7) and the resultant 5ASA levels in the AP chamber were higher than levels in the BL chamber.

Discussion

Previous work on 5ASA intestinal absorption has indicated that the drug is better absorbed in the upper than the lower intestine (Bondesen 1997). This has motivated the development of controlled release preparations for drug delivery to minimize systemic 5ASA absorption in the proximal intestine and to deliver more drug to diseased intestine in the lower bowel (Wikberg et al., 1997). The rat perfusion studies performed here confirm this region-dependent absorption. The higher metabolite level detected in the jejunum as compared with the ileum could be a function of regional differences in 5ASA permeability but might also be the result of regional differences in metabolic enzyme activity or metabolite transport capacity.

In situ perfusion studies demonstrate that intestinal 5ASA metabolism plays a key role in presystemic 5ASA elimination at a concentration of 0.075 mg/ml. At this concentration, more than 60% of the 5ASA loss from the intestinal lumen (11% of initial input concentration) is the result of intestinal metabolism with subsequent secretion of N-acetyl 5ASA into the intestinal lumen. Drug efflux from intestinal cells is not measurable in this system and is included in the calculation as unabsorbed drug. At low 5ASA perfusion concentrations, higher intestinal tissue metabolite levels as compared to plasma levels are likely the result of intestinal metabolism as opposed to transport of systemic metabolite into the tissue (Fig. 3). Concentration dependence of the lumenal metabolite appearance (Fig. 2) is indicative of either saturation of cytosolic NAT and/or saturation of enteroocyte mucosal metabolite secretion pathways. However, as intestinal perfusion concentrations of 5ASA increase, a larger fraction of 5ASA is absorbed into the systemic circulation (Fig. 1) and systemic metabolism plays a greater role in drug elimination as indicated by the higher plasma metabolite levels shown in Fig. 3.

The data in human intestinal cell monolayers was obtained over a broader concentration range than in the in situ work and provides evidence for carrier-mediated saturable transepithelial 5ASA transport at low AP concentrations. Carrier transport of 5ASA reaches saturation at a mucosal concentration of around 0.1 mg/ml and could be mediated by the recently identified intestinal organic anion transporter (Yabuuchi et al., 1998). Furthermore, the Caco-2 data show that secretory flux is greater than absorptive flux at low mucosal concentrations of 5ASA and that metabolite flux is strictly secretory, suggesting an asymmetric distribution of AP versus BL transporters. Secretory flux influences 5ASA metabolism by increasing AP metabolite levels to a greater extent from AP drug incubation than BL drug incubation (Fig. 6). This data indicates that, in addition to metabolic enzymes, saturation of carrier-mediated mucosal transport of 5ASA may limit the magnitude of metabolite appearance in the intestinal lumen. For 5ASA concentrations above 1 mg/ml, the 5ASA flux across the Caco-2 monolayer is independent of concentration, indicating that saturated carrier-mediated transport is dominated by passive diffusion. Transepithelial 5ASA transport at these high concentrations is more likely via the aqueous paracellular pathways rather than through lipid membrane permeation. Transcellular 5ASA transport via lipid membrane permeation is unlikely to contribute signifi-
Octanol-water and chloroform-water partition coefficients (log \(K_a\) 2.3, \(pK_a\) predominantly anionic (\(pK_a\) 4.8)) ZHOU ET AL. taken up at the BL side of the epithelia with elimination into the intestinal lumen. Excessive, for example in renal failure, both drug (#) and metabolite (?) may be excreted in the urine. When systemic drug and metabolite concentrations are significant. These more typical pathways are drawn across solid-line boundaries. Low lumenal 5ASA concentration ranges over which intestinal elimination may be significant. These more typical pathways are drawn across solid-line boundaries. The fraction of drug transported transcellularly and not metabolized or transported paracellularly enters the circulation where it can be metabolized by either the liver or kidney (as well as by other organ systems) and both drug and metabolite are excreted in the urine. When systemic drug and metabolite concentrations are excessive, for example in renal failure, both drug (#) and metabolite (?) may be taken up at the BL side of the epithelia with elimination into the intestinal lumen. These pathways, across dotted line-boundaries, may only be operative when other elimination pathways are compromised. Saturable metabolism of 5ASA by cytosolic NAT was observed in both the in situ and cell monolayer systems. The in situ results suggest and the cell monolayer studies (Fig. 6) confirm, that the \(N\)-acetyl 5ASA formed in intestinal epithelia is secreted in the mucosal direction only and that systemic metabolite is unlikely to be secreted from plasma into the intestinal lumen except at very high plasma concentrations. The finding is supportive of clinical data showing that \(N\)-acetyl 5ASA is not absorbed after lumenal administration and that i.v. administration of 5ASA results in essentially complete elimination in the urine as parent drug and metabolite (Myers et al., 1987; Bondesen et al., 1991). The capacity for intestinal secretion and metabolism of 5ASA is low as compared with renal excretion and hepatic metabolism, respectively, and the route of administration determines the elimination profile of 5ASA. Although there is an indication of reversible metabolism in the cell monolayer, the capacity for metabolite-to-parent drug conversion is low (Fig. 7), which is consistent with clinical observations (Meese et al., 1984). Projecting metabolism and transport observations in the Caco-2 cells and in situ studies to the clinical situation, cytosolic metabolism, and carrier-mediated transport of drug and metabolite should control drug absorption and elimination at low lumenal concentrations. Although the relative contributions of transcellular pathways have not been determined, paracellular absorption would be projected to dominate at high concentrations. The fact that controlled release 5ASA dosage forms, designed to target drug delivery to the lower bowel, provide for low lumenal drug concentrations (Christensen et al., 1990, 1993; Bondesen, 1997) indicates that saturable metabolism and transport in the intestine play important roles in drug therapy. The mediated unidirectional flux of \(N\)-acetyl 5ASA from the epithelia into the intestinal lumen provides a tool for separating intestinal and hepatic contributions to the \(N\)-acetylation of 5ASA. Subsequent to 5ASA uptake and metabolism by intestinal epithelia, the cells secrete the \(N\)-acetyl metabolite into the intestinal lumen. Absorbed drug that is not metabolized by the intestinal cells enters the circulation and is subject to hepatic metabolism and subsequent renal excretion. Systemic drug and metabolite are unlikely to enter intestinal cells even from i.v. injection (Shafii et al., 1982; Fischer et al., 1983) and intestinal secretion of systemic drug and metabolite is even more unlikely after oral absorption. Furthermore, \(N\)-acetylation of 5ASA by intestinal bacteria is minimal (Allgayer et al., 1989). Thus, lumenal and fecal metabolite levels represent a measure of intestinal metabolism, whereas urinary levels are a measure of systemic or hepatic metabolism. This mechanistic information may provide a link between 5ASA absorption and pharmacology by establishing a pivotal role for intestinal metabolism in the mechanism of action of 5ASA. Based on this tool for separating intestinal contributions to 5ASA elimination, data from previous studies can be revisited to assess the significance of intestinal metabolism and secretion in comparison with liver metabolism and renal secretion. A meal effect and a formulation effect study on 5ASA elimination are selected for illustration. In a food effect study, oral administration of 1 g 5ASA, formulated in 40 ml of an aqueous suspension, resulted in over 90% local and systemic bioavailability as assessed by cumulative urinary 5ASA (21.2%), \(N\)-acetyl 5ASA (57.1%), and fecal \(N\)-acetyl 5ASA (11.3%) elimination measurements (Yu et al., 1990). Projections for elimination contribution suggest that 11.3% of the absorbed drug can be accounted for by intestinal \(N\)-acetylation whereas liver metabolite accounts for 78.3% of systemic 5ASA elimination via urinary excretion. With concomitant food ingestion, the contribution of intestinal metabolism increased to 24.2% as indicated by cumulative fecal \(N\)-acetyl 5ASA measurements. Hepatic metabolism and urinary elimination of both 5ASA and \(N\)-acetyl 5ASA decreased by over 30% as a result of reduced rate and extent of ASA absorption into the systemic circulation in the presence of food. In comparison with meal effects on intestinal metabolism contributions to 5ASA elimination, intestinal \(N\)-acetylation of 5ASA plays a greater role in total body drug clearance when drug is administered as a delayed release dosage form. In a study of orally administered Mesalamine microgranules (Layer et al., 1995), systemic \(N\)-acetylation of 5ASA as measured by urinary excretion accounted for only 3.5% of the total 5ASA dose administered. Although the fecal content of \(N\)-acetyl 5ASA was not reported in this study, levels of 5ASA (64 \(\mu\)g/ml) and its metabolite (104 \(\mu\)g/ml) measured in the intestinal lumen projects the contribution of intestinal metabolism to at least 50% of total \(N\)-acetylation of 5ASA. Similar projections can be applied to results from other studies on sustained-release dosage forms of 5ASA (Christensen et al., 1990; Larouche et al., 1995; Yu et al., 1995). Typically, renal excretion of hepatic metabolite for sustained or delayed release 5ASA products accounts for 20 to 30% of
total drug elimination (Physician’s Desk Reference, 1997). Fecal contents include unabsorbed drug and intestinal metabolite, which accounts for over 50% of the total dose (prescription product package inserts for Pentasa and Asacol).

The unique transport and elimination patterns of 5ASA as assessed in this study (Fig. 8) may provide further information on the mechanism of action of this drug. Intracellular 5ASA uptake does not contribute to systemic drug absorption and the uncovering of significant intestinal 5ASA metabolism, and undirectional secretion of N-acetyl 5ASA from enterocytes into the intestinal lumen may help to provide resolution to a long-standing pharmacological research dilemma. Oral N-acetyl 5ASA has been shown to be ineffective, whereas intestinal metabolite tissue levels correlate with therapeutic effect (Easterbrook et al., 1998). Thus, 5ASA delivery and metabolism data as well as metabolite transport and distribution information gleaned from cell and tissue studies may serve to sort the relative contributions of drug and metabolite in clinical pharmacology and therapeutics.

References