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# Concentration Dependence of 5-Aminosalicylic Acid Pharmacological Actions in Intestinal Mucosa after Oral Administration of a pH-Dependent Formulation

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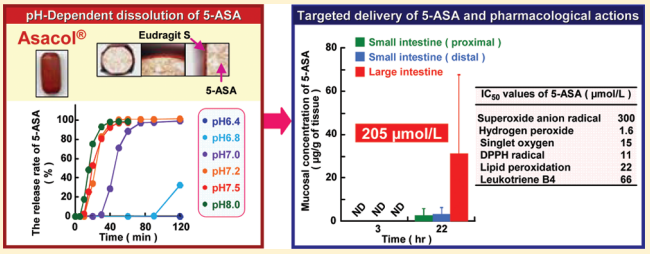
**ABSTRACT:** Asacol, a medication that delivers delayed release 5-aminosalicylic acid (5-ASA), is a useful therapeutic agent for inflammatory bowel disease (IBD), but the relationship between its pharmacological actions and intestinal concentrations has not been studied in detail. Therefore, our aim was to assess 5-ASA's pharmacological actions as a function of its concentration at its target site. We first evaluated 5-ASA's release profiles in vitro by the paddle method and found that Asacol starts to release 5-ASA at pH ≥ 7. Orally administered Asacol pharmacokinetic parameters were evaluated in dogs. Asacol's $T_{max}$ was much longer than that of the time-dependent release 5-ASA formulation. We also determined 5-ASA's distribution in the intestinal mucosa and found that it is effectively delivered there by Asacol. These results indicated that Asacol released 5-ASA in a pH-dependent manner, resulting in efficient delivery to the large intestine. We also compared the mucosal 5-ASA concentrations with the $IC_{50}$ values for scavenging free radicals or suppressing $LTB_4$ production. The 5-ASA concentration in the large intestine was higher than $IC_{50}$ values necessary to suppress inflammatory processes. We also report the release characteristics of Asacol and the targeted delivery of 5-ASA to affected sites in IBD patients.




**KEYWORDS:** asacol, 5-aminosalicylic acid, ulcerative colitis, inflammatory bowel disease, pH-dependent controlled release, mesalazine, mesalamine

## INTRODUCTION

Inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), is a general term for any condition involving chronic inflammation of the bowel wall with alternating periods of relapse and remission. UC is a chronic inflammation of the colon wall and rectum, whereas CD may involve inflammation in the gastrointestinal tract anywhere from the mouth to the anus, but mainly presents in the ileocecal region.[1] Although the trigger for an acute attack is not known, the pathophysiological mechanism of tissue damage in the active phase of IBD has been investigated.[2,3] It is widely believed that tissue damage in IBD results from abnormal mucosal immune reactions to bacterial products and other luminal factors that initiate an inflammatory cascade. The immunopathologies of CD and UC are characterized by distinct as well as by common pathogenetic factors such as dietary antigens and enteric commensal flora.[4,5]

Mucosal immune cell activation and the release of proinflammatory mediators come first leading to attraction of leukocytes to the injured site.[2,3,6,7] Leukotriene $B_4$ ($LTB_4$) is an immunomodulatory mediator and a highly potent chemoattractant for neutrophils, monocytes, and effector T cells.[8−11] Colonic mucosa from IBD patients exhibit increased production of $LTB_4$ compared to those of normal subjects.[12−16] $LTB_4$ also inhibits neutrophil apoptosis and increases the release of neutrophil mediators including free radicals, which may contribute to intestinal damage.[10] These findings indicate that scavenging free radicals and suppressing $LTB_4$ expression are important for inducing or maintaining remission in UC and CD.

IBD can be effectively treated with 5-aminosalicylic acid (5-ASA), known as mesalazine or mesalamine. The precise mechanism of action of 5-ASA is unknown, but may involve protecting the intestine from inflammation-mediated damage. Thus, 5-ASA has been reported to scavenge free radicals directly,[17,18] to inhibit leukotriene production,[19] and to inhibit inflammatory activity.[20−22]

The presumptive topical mode of action of 5-ASA suggests that it would be best to obtain a maximal concentration at the site of disease (the terminal ileum and colorectum). 5-ASA administered orally is, however, almost completely absorbed in the stomach and the proximal small intestine followed by its acetylation and excretion in urine.[23] For the purpose of overcoming this problem, several 5-ASA formulations, such as Asacol and time-dependent release 5-ASA formulation, have been developed to release the substance into the gut lumen.

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A time-dependent, prolonged-release tablet contains 5-ASA in microgranules coated with ethylcellulose for release into the intestine.[24] Asacol, a delayed-release tablet, is coated with an acrylic based resin, Eudragit S, that dissolves at pH values ≥ 7, releasing 5-ASA in the terminal ileum and colon.[17,25] However, detailed intestinal delivery patterns of 5-ASA after oral administration of Asacol and whether the 5-ASA concentration released from Asacol is sufficient for its intended action have not been directly determined.[26] Although the concentration of 5-ASA in large intestinal mucosa has been investigated in humans by determining levels in 5-ASA in biopsies, 5-ASA's dose−response properties are unknown.[27] This arises, in part, from 5-ASA's rapid conversion to *N*-acetyl-5-ASA by *N*-acetyltransferase[23] upon 5-ASA uptake into the large intestinal mucosa. To eliminate this complication, we evaluated the mucosal concentration of 5-ASA from Asacol using canids, which lack the gene encoding N-acetyltransferase[28] and have an intestinal pH pattern similar to that of humans.[29] Thus, dogs are suitable for evaluating the colon-specific delivery of pH-dependent release preparations such as Asacol.

In the present study, the dissolution characteristics of 5-ASA from Asacol were examined using phosphate buffers that induced pH changes along the gastrointestinal (GI) tract. Furthermore, the mucosal concentration of 5-ASA released from Asacol in intestine was determined in dogs and compared with the $IC_{50}$ values of 5-ASA for free radical scavenging and suppressing $LTB_4$ production.

## MATERIALS AND METHODS

**Materials.** Asacol 400 mg tablets were obtained from Tillotts Pharma AG (Ziefen, Switzerland). Pentasa 250 mg tablets (Kyorin Pharmaceutical Co., Ltd., Tokyo, Japan) were used as a time-dependent release 5-ASA formulation (TDR formulation). 5-ASA and 4-aminosalicylic acid (used as the internal standard for the assay) were purchased from Acros Organics (Geel, Belgium). Arachidonic acid, hydrogen peroxide, and scopoletin were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Calcimycin, 2-deoxy-D-ribose, superoxide dismutase, and xanthine oxidase were purchased from Sigma-Aldrich Japan K.K. (Tokyo, Japan). 1,1-Diphenyl-2-picrylhydrazyl (DDPH) and hypoxanthine were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). 2-Methyl-6-phenyl-3,7-dihydroimidazo[1,2-a]pyrazin-3-one (CLA) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). All chemicals were analytical reagent or special reagent grade.

**Animals.** Nine-month-old male beagles (each weighing approximately 10 kg) were purchased from KitayamaLabes Co., Ltd. (Nagano, Japan). Six-week-old male Sprague−Dawley rats were purchased from Charles River Laboratories Japan, Inc. (Kanagawa, Japan). All animals were treated in accordance with the guidelines for animal experimentation stipulated by the ethics committee of Zeria Pharmaceutical Co., Ltd. All animals were fed and given free access to water while acclimatizing during the experiment.

**pH-Dependent Dissolution of 5-ASA from Asacol.** Asacol dissolution tests were performed using phosphate buffers adjusted to pH 6.4, 6.8, 7.0, 7.2, 7.5, or 8.0, equilibrated to 37 °C at 100 rpm by the paddle method. Aliquots were withdrawn at the times indicated in Figure 1. 5-ASA concentrations were determined by high-performance liquid chromatography (Alliance HPLC, Waters Co., Milford, MA) using a reverse phase column (Unison UK-Phenyl, 150 × 4.6 mm internal diameter, 3 μm silica particles; Imtakt Co., Kyoto, Japan) with UV detection (230 nm). The mobile phase consisted of 22 mM $KH_2PO_4$, 5.6 mM octanesulfonic acid (pH 2.0 adjusted with $H_3PO_4$), and methanol (9:1, v/v), delivered at 0.8 mL/min.

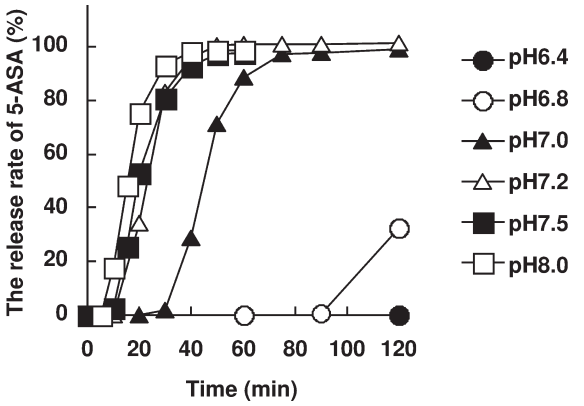


**Figure 1.** Effect of pH on the dissolution medium on 5-ASA release from Asacol. pH 6.4 (closed circles), pH 6.8 (open circles), pH 7.0 (closed triangles), pH 7.2 (open triangles), pH 7.5 (closed squares), and pH 8.0 (open squares) of the dissolution medium.

**Absorption Kinetics of 5-ASA in Dogs.** Six male beagles were randomly assigned to one of two crossover experiments with a washing out period of one month. Asacol (one tablet containing 400 mg of 5-ASA) or a TDR formulation (two tablets containing a total of 500 mg of 5-ASA) were orally administrated to each dog. Blood samples were collected at over 48 h. Plasma was immediately prepared by centrifuging of blood samples at 2500*g* for 10 min and stored at −30 °C.

**Distribution of 5-ASA in Colonic Mucosa.** Twenty-four male beagles were randomly assigned to four groups. Asacol (one tablet containing 400 mg of 5-ASA) or the TDR formulation (two tablets containing a total of 500 mg of 5-ASA) were orally administrated to each dog. Animals in two groups were euthanized 3 h after administration, and the other two groups were euthanized at 22 h. Immediately after euthanization, the intestines were excised and rinsed with physiological saline. Mucosa representing proximal and distal small and large intestines was scraped from 20 cm long segments from 10 cm distal to the pyloric end, and from tissue proximal, and distal to cecum.

**Measurement of 5-ASA in Tissues.** The mucosal samples were weighed and homogenized in 0.05 M phosphate buffer (pH 7.4) to 10% (w/v), sonicated at 40 W for 2 min, and centrifuged at 500 rpm for 10 min. Measures of 10 μL of propionic anhydride, 500 μL of methanol, and 20 μL of 100 μg/mL 4-aminosalicylic acid (internal standard) were added to supernatants (400 μL) and mixed well by vortexing. These mixtures were allowed to stand at room temperature for 30 min and centrifuged, and the supernatant passed through Ultrafree-MC (0.22 μm; Millipore Corp., Billerica, MA) filters. A sample of 20 μL of sample solution was injected into an HPLC system to determine the mucosal concentration of 5-ASA. Measures of 10 μL of propionic anhydride, 200 μL of 0.05 M phosphate buffer (pH 7.4), 500 μL of methanol, and 20 μL of 100 μg/mL 4-aminosalicylic acid (internal standard) were added to plasma samples (200 μL) and mixed well by vortexing. The 20 μL samples were analyzed by HPLC to determine 5-ASA plasma concentrations. HPLC conditions: Waters Alliance HPLC (Waters Co., Milford, MA); Waters 2475

multifluorescence detector, 315 nm excitation, 430 nm emission; column, Senshu Pak PEGASIL-B C4 (5 $\mu$m, 4.6 mm i.d. × 150 mm; Senshu Scientific Co., Ltd., Tokyo, Japan); column temperature, 40 °C; mobile phase, distilled water, acetonitrile, isopropanol, and acetic acid (990:60:30:10); and flow rate, 1 mL/min. The analytical and pretreatment methods were validated over a nominal linear range of 50−10000 ng/mL 5-ASA in plasma and 25−5000 ng/mL in mucosal homogenate supernatants.

**Reactive Oxygen Species (ROS) Scavenging by 5-ASA.** Superoxide anion radicals generated by a hypoxanthine-xanthine oxidase system were quantified using Cypridina luciferin analogue (CLA)-enhanced chemiluminescence. The reaction was initiated by the addition of xanthine oxidase to the solution containing CLA, hypoxanthine, and 5-ASA. Chemiluminescence was measured using an ARVOsx multilabel counter (PerkinElmer Life Sciences, Inc., Boston, MA). Hydrogen peroxide ($H_2O_2$) was measured using the scopoletin method.[30] Horseradish peroxidase and scopoletin were added to a solution containing $H_2O_2$ and 5-ASA, and fluorescence extinction was measured. Absorbance was measured at 550 nm using a microplate reader (Tecan Austria GmbH, Salzburg, Austria). Singlet oxygen generated by $H_2O_2$ and sodium hypochlorite was quantified using CLA-enhanced chemiluminescence. The reaction was initiated by the addition of sodium hypochlorite to a solution containing $H_2O_2$, CLA, and 5-ASA. Scavenging activity using 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH-radical) was determined by monitoring the decreased absorbance (517 nm) of DPPH−ethanol. The suppressive effect on lipid peroxidation in liver microsomes was determined as thiobarbituric acid reactive substances[31] by measuring the absorbance at 535 nm.

**Preparation of Rat Polymorphonuclear Leukocytes (PMN).** Polymorphonuclear leukocytes (PMN) were prepared from rat blood by a published method.[32] PMNs were suspended ($2 \times 10^7$ cells/mL) in $Ca^{2+}$-free Hank's balanced salt solution (pH 7.65).

**Suppressive Effect of 5-ASA on $LTB_4$ Generation from PMNs.** The suppressive effect of 5-ASA on $LTB_4$ generation from PMNs was examined as described.[33] The reaction was initiated by the addition of arachidonic acid and calcimycin to a preincubated (37 °C) PMN suspension containing 5-ASA. The reaction was performed for 10 min, terminated by submersion in ice-cold water, and centrifuged. $LTB_4$ was determined using the $LTB_4$ ELISA kit (R&D Systems, Inc., Minneapolis, MN).

**Determination of $IC_{50}$ Value.** To determine $IC_{50}$ values the logistic curve was fit to the data sets generated by the assays described above by the nonlinear squares method using a SAS System Version 8.2 (SAS Institute Inc., Cary, NC) to obtain estimates of $IC_{50}$ values.

**Determination of Kinetic Parameters.** All pharmacokinetic parameters were calculated using WinNonlin Version 4.1 software (Pharsight Inc., Mountain View, CA). $C_{max}$ represents the maximum drug concentration, $T_{max}$, the time to reach the $C_{max}$, and $T_{first}$ the time at which the drug was first detected. These values were obtained as directly measured values. The elimination half-life ($T_{1/2}$) was calculated from the slope of the curve generated by plotting the logarithm of plasma concentration versus time at the four end points. The areas under the plasma concentration−time curve ($AUC_{0-t}$) were calculated by the trapezoidal method.

## RESULTS

**pH-Dependent Dissolution of 5-ASA from Asacol.** The dissolution characteristics of 5-ASA from Asacol were examined with the paddle method using dissolution media with different pH values to simulate gastrointestinal (GI) fluids. As shown in Figure 1, medium-range pH values markedly affected 5-ASA release. Thus, at pH 7.0, over 90% of the dose amount of 5-ASA was released within 75 min. In addition, the time to release of 5-ASA became shorter as pH increased, and at pH > 7.0, the release rate was strongly enhanced.

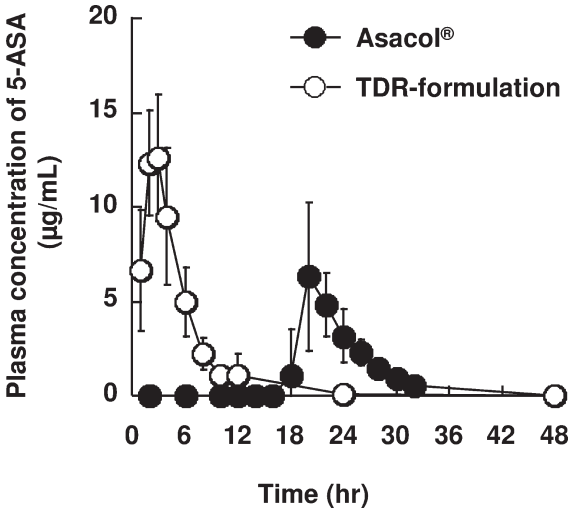


**Figure 2.** Plasma concentration kinetics of 5-ASA after oral administration of Asacol (closed circles) and TDR formulation (open circles) in dogs. Asacol and TDR formulation were administered as one tablet (400 mg) and two tablets (500 mg total), respectively. Each point and vertical bar represents the mean ± SD of six different experiments.

**Table 1. Pharmacokinetic Parameters of 5-ASA after the Oral Administration of Asacol or a TDR Formulation to Dogs[a]**

| parameters | Asacol | TDR formulation |
|---|---|---|
| $T_{first}$ (h) | 19.7 ± 1.5 | 1.0 ± 0.0 |
| $T_{max}$ (h) | 20.7 ± 1.0 | 2.5 ± 0.5 |
| $C_{max}$ ($\mu$g/mL) | 6.94 ± 3.04 | 13.2 ± 3.4 |
| $AUC_{last}$ ($\mu$g·h/mL) | 40.6 ± 17.0 | 68.8 ± 18.9 |
| $t_{1/2}$ (h) | 2.6 ± 0.9 | 2.5 ± 0.7 |

[a] Mean ± S.D. of six dogs.

**In Vivo Absorption Kinetics of 5-ASA after Oral Administration of Asacol or a TDR Formulation.** Either Asacol or the TDR formulation were orally administered in the two crossover experiments to beagles at a dose of 400 or 500 mg/body, respectively. Figure 2 and Table 1, respectively, present the 5-ASA plasma concentration−time curve and pharmacokinetic parameters. The mean $T_{first}$ and $T_{max}$ were, respectively, 19.7 and 20.7 h after oral administration of Asacol, and 1 and 2.5 h after oral administration of TDR formulation. Asacol's $C_{max}$ and $AUC_{last}$ values were, respectively, 6.94 $\mu$g/mL and 40.6 $\mu$g·h/mL, which were lower than those of TDR formulation. $T_{1/2}$ was comparable for both drugs.

**Distribution of 5-ASA in Intestinal Mucosa after Administration of Asacol or a TDR Formulation.** The distribution of 5-ASA in proximal small intestinal, distal small intestinal, and large intestinal mucosa was measured after oral administration of Asacol to establish the site-specific delivery of 5-ASA. At 3 h, 5-ASA was not detected in any intestinal site, but 5-ASA was detected in all sites after 22 h, with concentrations in mucosa of proximal small intestine, distal small intestine, and large intestine of 2.74, 3.24, and 31.4 $\mu$g/g of tissue, respectively (Figure 3). The

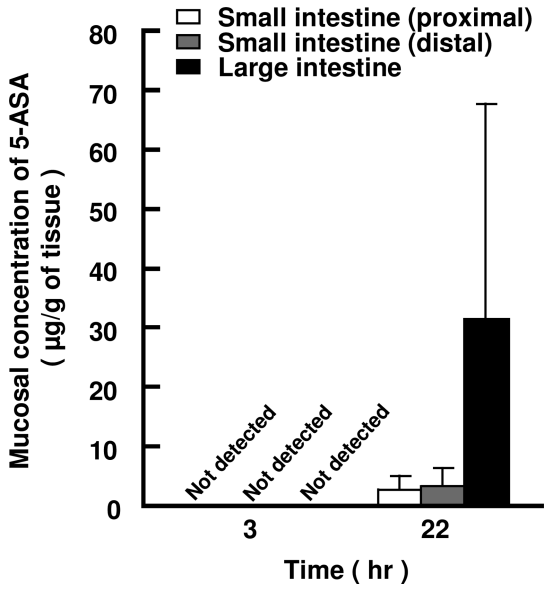


**Figure 3.** Mucosal concentrations of 5-ASA in the proximal and distal small intestine and in the large intestine after oral administration of Asacol to dogs. Asacol was administered as one tablet (400 mg). The vertical bar represents the mean + SD of six different experiments.

**Table 2. Mucosal-to-Plasma Concentration Ratio (M/P Ratio) of 5-ASA after the Oral Administration of Asacol (400 mg) or a TDR Formulation (500 mg) to Dogs[a]**

| | Asacol | TDR formulation |
|---|---|---|
| small intestine (proximal) | 0.57 | 0.71 |
| small intestine (distal) | 0.67 | 2.0 |
| large intestine | 6.5 | 2.7 |

[a] Asacol groups were euthanized at 22 h after administration, and TDR formulation groups were euthanized at 3 h. Mean of six dogs.

mucosa-to-plasma concentration ratio (M/P ratio) of Asacol and the TDR formulation is shown in Table 2. The M/P ratio (6.5) was highest in large intestine after Asacol administration and was 0.71−2.7 after the administration of TDR formulation.

**Effects of 5-ASA on ROS Scavenging and Inhibition of Lipid Peroxidation by 5-ASA.** We next examined the effect of 5-ASA on ROS (reactive oxygen species) and free radicals. 5-ASA decreased the detection of superoxide anion radicals that were generated hypoxanthine-xanthine oxidase, with an $IC_{50}$ value of 300 $\mu$M (Figure 4, Table 3). 5-ASA scavenged hydrogen peroxide with an $IC_{50}$ value of 1.6 $\mu$M and singlet oxygen with an $IC_{50}$ value of 15 $\mu$M (Figure 4, Table 3). Moreover, 5-ASA reduced DPPH-radical detection and suppressed lipid peroxidation with $IC_{50}$ values of 14 and 22 $\mu$M, respectively (Table 3).

**Suppression of $LTB_4$ Generation from PMNs by 5-ASA.** The suppression of $LTB_4$ generated by PMNs was examined using 5-ASA at concentrations from 1 to 1000 $\mu$M. 5-ASA suppressed $LTB_4$ generation from PMNs with an $IC_{50}$ value of 66 $\mu$M (Figure 5, Table 3).

## DISCUSSION

5-ASA is an essential therapeutic agent for treating IBD. Orally ingested, uncoated 5-ASA is almost completely absorbed in the stomach and the proximal small intestine.[23] Therefore, several methods have been used to reduce this proximal absorption. In Asacol, a delayed release preparation, 5-ASA is coated with a pH-dependent acrylic-based resin, Eudragit S.[33] In human studies, Asacol released 5-ASA into the terminal ileum and sites farther

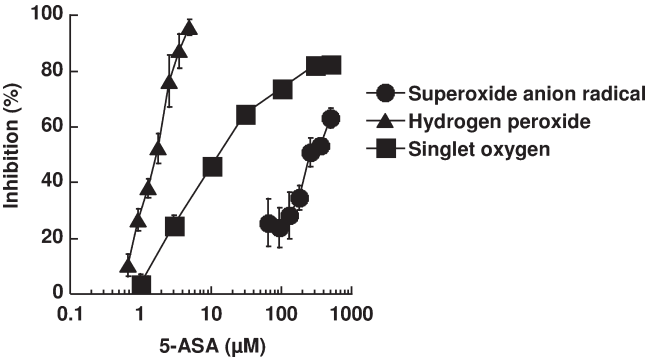


**Figure 4.** Inhibitory effect of 5-ASA on scavenging by reactive oxygen species. Each point and vertical bar represent the mean ± SE from four individual experiments performed in triplicate.

**Table 3. $IC_{50}$ Values of 5-ASA for Reactive Oxygen Scavenging, Free Radical Reduction Effect, and Suppression Effect of $LTB_4$**

| | 5-ASA ($\mu$M) |
|---|---|
| superoxide anion radical[a] | 300 |
| hydrogen peroxide[a] | 1.6 |
| singlet oxygen[a] | 15 |
| DPPH radical[a] | 14 |
| lipid peroxidation[a] | 22 |
| LTB4[b] | 66 |

[a] Data shown are the mean from four individual experiments performed in triplicate. [b] Data shown are the mean from seven individual experiments performed in triplicate.

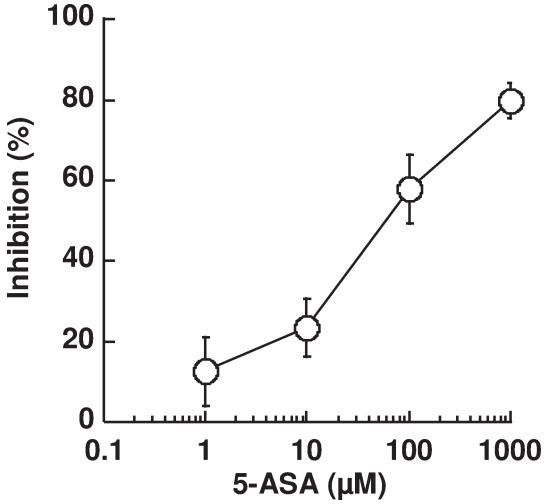


**Figure 5.** Inhibitory effect of 5-ASA on $LTB_4$ generation from rat PMNs. Each point and vertical bar represents the mean ± SE from seven individual experiments performed in triplicate. Generation of $LTB_4$ (open circles) is a function of the concentration of 5-ASA in the medium.

down the GI tract.[27] However, with the exception of the present report, the detailed intestinal delivery patterns of 5-ASA, including distribution to small and large intestines after oral administration of Asacol, have not been studied in detail. In their review of 5-ASA formulations used for UC therapies, Lichtenstein and Kamm point out that plasma pharmacokinetics can reveal systemic exposure and that mucosal biopsies definitively establish colonic distribution and may also predict clinical efficacy. However, these data must be interpreted cautiously because of considerable intersubject variability and other confounding factors.[26]

The 5-ASA dissolution characteristics from Asacol were examined in dissolution media at several different pH values, mimicking the GI tract environment. As shown in Figure 1, medium-range pH values notably affected 5-ASA release, so that Asacol was rapidly released pH ≥ 7.0 or higher, with slow release at pH 6.8 and no release at pH 6.4. Moreover, the time until release of 5-ASA was much shorter at higher pH. The intestine's luminal pH is affected by mucosal bicarbonate and lactate production, bacterial fermentation of carbohydrates, mucosal absorption of short chain fatty acids, and possibly intestinal transit. The reduction in luminal pH in IBD arises from alterations in these factors resulting from mucosal disease and dietary changes. However, the distal small bowel pH in IBD patients ranges from 6.8 to 8.3,[34] which causes 5-ASA release from Asacol. In contrast, UC patients' intestinal transit times are not reduced but are similar to those of healthy controls.[34] Thus, this suggests that Asacol did not release 5-ASA in proximal intestine under these conditions.

To clarify whether this pH-dependent release affects pharmacokinetic parameters, we administered Asacol or a TDR formulation, commonly used controlled-release preparations, to dogs, and compared pharmacokinetic parameters. Our results indicate that Asacol releases 5-ASA to a much lower extent in the small intestine than does the TDR formulation, consistent with the pH-dependency data. Thus, Asacol more effectively sequesters 5-ASA thereby reducing its systemic absorption compared with the TDR formulation.

Subsequently, we showed here that 5-ASA concentrations in the large intestinal mucosa were approximately 10 times higher than those determined for the proximal and distal intestinal mucosa. This result was consistent with 5-ASA absorption kinetics showing that it was first detected after 19.7 h. Sutton reported the intestinal pH profile of dogs, in which pH values were 5−7.6 in the duodenum, 6.2−7.3 in the jejunum, and 6.6−7.9 in the ileum. The pH of human duodenum, jejunum, and ileum, similarly, are 5−7.8, 5−7, and 7.0, respectively.[29] Moreover, dogs lack *N*-acetyltransferase activity due to absence of NAT genes[28] Thus, dogs permit evaluation of colon-specific, pH-dependent 5-ASA delivery by preparations such as Asacol. Our use of a canine model supports this assumption and establishes that 5-ASA was mainly and efficiently released in the distal small intestine.

Here, we were also able to demonstrate that Asacol efficiently delivered 5-ASA to the large intestine. The therapeutic effect of 5-ASA was shown to result from a local, rather than a systemic effect on the mucosa;[35,36] however, 5-ASA's mechanism of action remains a mystery. Several mechanisms have been proposed, including scavenging of reactive oxygen metabolites,[22] inhibition of leukocyte chemoattractants[36] and peroxisome proliferative activated receptor-$\gamma$ (PPAR-$\gamma$) agonistic action,[37] inhibition of the inflammatory actions of interleukin 1 and tumor necrosis factor,[19,38] and inhibition of the production of prostaglandins and leukotrienes.[39] All of these mediators and mechanisms are likely to participate in the activation of an inflammatory cascade documented to exist in IBD patients and the consequent pathophysiologic progression in their intestinal walls.[11] The presumptive topical mode of action of 5-ASA protects against inflammation-mediated damage as described above indicates that it would be best to deliver a maximal concentration at the site of disease.

In this study, based on the assumption that tissue specific gravity = 1 g/mL, the concentration of 5-ASA in large intestinal mucosa was estimated to be 205 $\mu$M (31.4 $\mu$g/g of tissue), which was higher than $IC_{50}$ values, indicating that Asacol delivered enough 5-ASA to the large intestine to exert pharmacological actions relevant for the suppression of inflammatory processes. In clinical practice, the doses of Asacol are 1.2−4.8 g/day and 1.2−2.4 g/day for inducing remission and maintaining remission, respectively, for the maintenance of remission. The usual dosage is 1−3 Asacol 400 mg tablets taken three times a day.[40,41] Assuming linear pharmacokinetics and no species differences, based on a body weight (dog, about 10 kg; human, about 60 kg), the 5-ASA concentrations in large intestinal mucosa extrapolated from these clinical doses were estimated as 34.2−137 $\mu$M. In fact, when we compared these data with clinical values, 5-ASA and *N*-acetyl 5-ASA's total AUC (314.37−406.38 nmol·h/mL) at a clinical dose of 2400 mg[42] and 5-ASA's AUC (265.12 nmol·h/mL) at the 400 mg canine dose, were nearly equivalent. These results indicate a reciprocal relationship between clinical dose-AUC and body weight, thus supporting assumptions based on body weight. Therefore, the 5-ASA delivered to large intestine from Asacol plays an important role for the induction and maintenance of remission in patients with UC, and these effects might stem from scavenging free radicals and suppression of $LTB_4$ production.

## ■ CONCLUSIONS

In conclusion, we demonstrate here that, after administration of Asacol to dogs, the release of 5-ASA was substantially later and the total systemic exposure was lower than determined for the TDR formulation. These finding could decrease the potential for unexpected side effects. Furthermore, we showed that Asacol efficiently delivered 5-ASA to the large intestine at concentrations in the intestinal mucosa sufficient to inhibit essential inflammatory pathways. Taken together, these results indicate that Asacol is an efficient oral formulation for targeting the colon of patients suffering from IBD.

## ■ AUTHOR INFORMATION

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