# Comparative Analysis of the In Vitro Prosecretory Effects of Balsalazide, Sulfasalazine, Olsalazine, and Mesalamine in Rabbit Distal Ileum

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**Background:** The aminosalicylates remain foundation therapy for mild-to-moderate ulcerative colitis. Pro-drug 5-aminosalicylic acid (5-ASA; mesalamine) formulations have been developed to prevent 5-ASA from the proximal absorption and release of mesalamine, to decrease inflammation, and to improve colonic absorption. Clinically, pro-drugs such as olsalazine have been associated with dose-dependent diarrhea, which was likely secondary to ileal secretion induced by the azo linkages, in 17% of patients. The present study tested the hypothesis that the use of all compounds with azo linkages leads to increased secretion.

**Methods:** Intestinal tissue was randomly assigned to serve as controls or to receive brush border addition of equimolar concentrations of the compounds, and the change in short-circuit current was measured.

**Results:** Mesalamine did not induce secretion at any dose. Mean equivalent doses (0.1 to 10 mM) of balsalazide (range, 6.3 ± 1.5 to 16.7 ± 1.3 $\mu A/cm^2$), olsalazine (range, 2.0 ± 1.0 to 7.0 ± 2.1 $\mu A/cm^2$), and sulfasalazine (3.2 ± 1.1 to 6.2 ± 1.5 $\mu A/cm^2$) significantly stimulated ($P < 0.001$) secretion. The values for the effective dose that is half the maximal dose for secretion induced by sulfasalazine, olsalazine, and balsalazide were 0.4, 0.7, and 0.9 mM, respectively.

**Conclusions:** This study is the first to demonstrate that the use of pro-drugs with azo bonds leads to increased ileal secretion at equimolar concentrations of 5-ASA. Physicians should use caution when providing higher doses of the pro-drug forms of 5-ASA to their patients, as this could lead to increased diarrhea.

**Key Words:** balsalazide, mesalamine, olsalazine, sulfasalazine, ulcerative colitis

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Ulcerative colitis is an idiopathic, chronic inflammatory disease of the colon with the requisite for both short-term and long-term medical therapy to induce and maintain remission.[1,2] The first-line therapies for the induction and maintenance of remission in patients with mild-to-moderate ulcerative colitis are aminosalicylates, including mesalamine formulations and the pro-drugs sulfasalazine, olsalazine, and balsalazide.[3–9] The beneficial effects of these drugs are attributed to 5-aminosalicylic acids (5-ASAs; mesalamine). Unlike mesalamine, the pro-drugs sulfasalazine, olsalazine, and balsalazide all have an azo linkage, allowing the compound to pass through the small intestine unchanged to arrive in the colon where they are metabolized by colonic bacterial azoreductase to release 5-ASA (Fig. 1, Table 1).[10–13]

However, clinical reports have indicated an increase in diarrhea in some patients receiving the pro-drug olsalazine, which has been attributed to the azo bond linkage.[12–15] Furthermore, we[14] and others[15,16] previously showed that olsalazine and sulfasalazine, but not 5-ASA, stimulated active anion secretion and inhibited NaCl absorption in the small intestine. No effects were observed in the colon. These observations are consistent with those of earlier studies that showed an increase in ileostomy output in subjects receiving olsalazine.[12] These actions could underlie the diarrhea-promoting effects of azo bond linkage compounds, complicating and limiting their usage for the treatment of inflammatory bowel disease.

Most recently, clinical trials have shown that a dose of 6.75 g of balsalazide daily (equal to about 2.4 g of 5-ASA) is sufficient to get an "optimal" response.[11] However, we have observed in clinical practice that patients develop diarrhea when the dose is increased. We hypothesized that the highest

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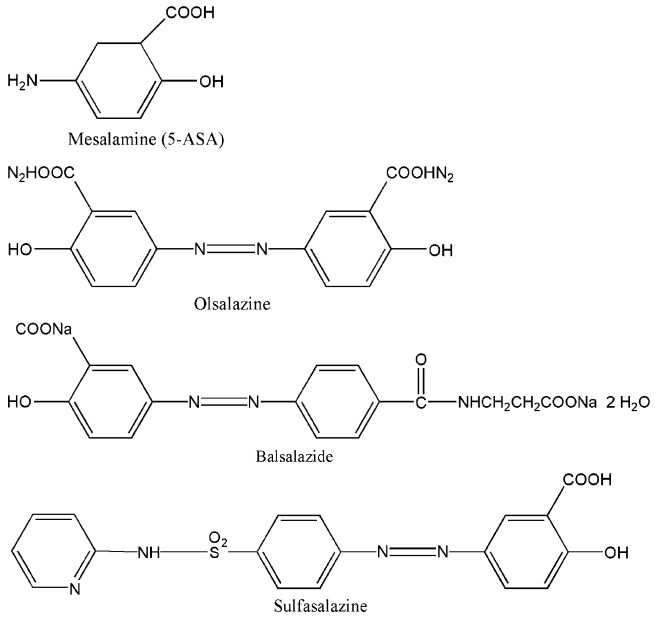


**FIGURE 1.** Chemical structures of compounds.

doses, above 6.75 g daily, also produce small-bowel secretion secondary to the azo bond, which is similar to that observed with olsalazine. No studies, to date, have tested comparable concentrations of each of these therapeutic agents for the induction of secretion. Therefore, the present study was designed to test the hypothesis that, in the rabbit ileum, equimolar concentrations of pro-drugs with an azo bond will induce secretion in a dose-dependent manner.

## MATERIALS AND METHODS

### Ion Transport Measurements

The effects of mesalamine (Asacol; Procter & Gamble Pharmaceuticals, Cincinnati, Ohio), balsalazide (Colazal; Salix Pharmaceuticals, Inc., Raleigh, NC), olsalazine (Dipentum; Pharmacia & UpJohn Co., Kalamazoo, Mich), and sulfasalazine (Sigma, St. Louis, Mo) on intestinal electrolyte transport were assessed in modified Ussing Chambers (VCC-600; Physiologic Instruments Inc., San Diego, Calif). New Zealand white male rabbits weighing 2 to 3 kg were killed according to Institutional Animal Care and Use Committee guidelines using an overdose of pentobarbital. Segments of the distal ileum (30 to 40 cm long) proximal to the ileal-cecal valve were rapidly excised, quickly removed from the abdominal cavity, and rinsed in ice-cold oxygenated Kreb's buffer (140 mM Na, 119.8 mM Cl, 25 mM $HCO_3$, 12 mM Mg, 1.2 mM Ca, 4.8 mM K, 2.4 mM $HPO_4$, and 0.4 mM $H_2PO_4$, pH 7.4). Segments were cut longitudinally along the mesenteric border and were stripped of their muscularis by blunt dissection. Segments of 10 to 15 cm were cut into squares of 2 to 3 cm, were mounted in modified Lucite Ussing (Physiological Instruments, Inc., San Drego, CA) chambers (1.13 $cm^2$ exposed surface area), and were bathed in a modified Kreb's solution. Tissue was maintained at 37°C by a circulating waterbath and was continuously gassed with 5% $CO_2$-95% $O_2$. Glucose (10 mM) was added to the serosal side, and mannitol (10 mM) was added to the brush border side during equilibration. Baseline transmural short-circuit current (Isc), resistance, and potential differences were measured after a 30-minute equilibration period by applying Ohm's law.

### Dose Response

After 30 minutes of equilibration, mesalamine, balsalazide, olsalazine, and sulfasalazine were added to the mucosal bath, ranging in dose from 0.001 to 10 mM (doses of 0.001 to 0.1 mM, n = 4; doses of 0.1 to 10 mM, n = 12). The tissue Isc response was recorded, and the maximal change was calculated. Each calculation was corrected for the exposed mucosal area. At the end of each experiment, D-glucose (10 mM) was added to the mucosal bath to validate the viability of the tissue. If the response was $<20$ $\mu A/cm^2$, the experimental set was rejected.

**TABLE 1.** Comparison Between 5-ASA Formulations

| Generic Name | Proprietary Name | Site of Delivery | Daily Dose (g) | Equivalent to 5-ASA (g) | Concentration of 5-ASA in Lumen | Reference |
|---|---|---|---|---|---|---|
| Mesalamine | Asacol | Terminal | 1.6–4.8 | 2.4 | 23.3 mM | 1 |
| | Pentasa | Illeum* | | | | |
| | Salofalk | Colon† | | | | |
| Balsalazide | Colazide | Colon | 2.25–6.75 | 2.4 | 15.9 mM | 11, 19, 28 |
| Disodium | Colazal | | | | (0.7–2.4 g 5-ASA) | |
| Olsalazine | Dipentum | Colon | 2–3 | 2–3 | 23.7 mM | 29, 30 |
| Sulfasalazine | Azulfidine | Colon | 4–6 | 1.6–2.4 | 12.6 mM | 19, 28 |
| | | | | | (0.8–1.6 g 5-ASA) | |

*This is dependent on formulation.
†It can also be delivered to duodenum, jejunum, ileum (Pentasa).

## Statistics

The data are presented as the mean ± SEM. Electrophysiology measurements were compared utilizing two-way analysis of variance comparing dose[7] and treatment.[5] When a significant effect existed, comparisons were completed using the Fisher protected least squared difference post hoc analysis. Statistics were performed using SAS (version 8.2; SAS Institute Inc., Cary, NC). Statistical significance was defined as $P \leq 0.05$.

# RESULTS

## Baseline Resistance, Isc, and Potential Difference

As expected after an equilibration period, neither transmural resistance, nor transmural Isc or potential difference were altered at baseline levels between any of the treatments (Table 2).

## Secretion-induced Ion Transport Following Treatment

Figure 2 shows the secretion-induced ion transport in controls and following treatment with mesalamine, balsalazide, olsalazine, or sulfasalazine. As shown in Figure 2, mesalamine did not stimulate secretion compared with the control. Balsalazide, olsalazine, and sulfasalazine all significantly stimulated ($P < 0.001$) secretion following addition to the mucosal reservoir.

## Dose-Response Isc Measurements

Sulfasalazine administered at doses between 0.001 and 10 mM significantly induced an 8-fold change in ion transport activities compared with controls and mesalamine-treated animals ($P < 0.001$). Olsalazine (Fig. 3) and balsalazide (Fig. 4) significantly induced ($P < 0.001$) a concentration-dependent induction of ion transport activity between 0.001 and 10 mM compared with controls and mesalamine-treated animals. Additionally, these 2 compounds induced a 7-fold and 17-fold change, respectively, in Isc following the addition of balsalazide and olsalazine concentrations of 10 mM. The values for the effective dose that is half the maximal dose (ED50) were calculated for compounds that induced secretion (sulfasalazine, 0.4 mM; olsalazine, 0.7 mM; and balsalazide 0.9 mM).

**TABLE 2.** Basal Ion Transport Activity

| Treatment | Resistance ($\Omega/cm^2$) | Isc ($\mu A/cm^2$) | Potential Difference ($mV/cm^2$) |
|---|---|---|---|
| Control | 48.8 ± 5.4 | 34.3 ± 12.8 | 0.48 ± 0.66 |
| Mesalamine | 50.8 ± 4.5 | 35.7 ± 10.7 | 1.73 ± 0.55 |
| Balsalazide | 46.2 ± 6.4 | 35.3 ± 15.1 | 1.45 ± 0.78 |
| Olsalazine | 45.2 ± 5.3 | 46.4 ± 12.4 | 1.65 ± 0.64 |
| Sulfasalazine | 38.9 ± 5.9 | 40.4 ± 13.9 | 1.52 ± 0.72 |

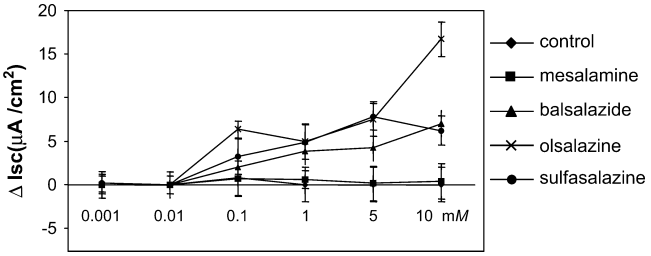


**FIGURE 2.** Pro-drug compounds dose dependently increase ion transport.

# DISCUSSION

This study demonstrates that the azo-bond pro-drugs balsalazide, olsalazine, and sulfasalazine have similar effects on ion transport activity. This effect is not observed with mesalamine but occurs with pro-drugs at concentrations (0.1 to 10 mM) that are reached clinically within the intestinal lumen at currently recommended doses (12 to 24 mM; Table 1). Previous studies have demonstrated that olsalazine stimulates Isc, a measure of anion secretion, with an ED50 of 0.3 mM when applied to the luminal side. The effect was far less pronounced when the compound was added to the serosal side and was not associated with changes in cyclic nucleotide levels.[14] Sulfasalazine also stimulated intestinal secretion with an approximate ED50 value of 7 to 8 mM. The data from the current study agree with those reported in the literature and support the clinical evidence that the effects that an azo bond produces are associated with ileal secretory effects, which can lead to diarrhea. The current study observed that balsalazide also has similar effects on Isc in the range of 0.1 to 10 mM. The colonic mucosa was not investigated in the current study because azo-bond compounds do not seem to affect electrolyte transport in this region. In a previous report from our laboratory[14] and in this study, mesalamine or 5-ASA had little or no effect on colonic ion transport. In the previous study, rabbit mucosa was exposed to an azo compound, which increased the net efflux of sodium and chloride. Clinically, this manifests as greater-than-normal levels of fluid and electrolytes in the proximal colon, and a diarrheal type response in the

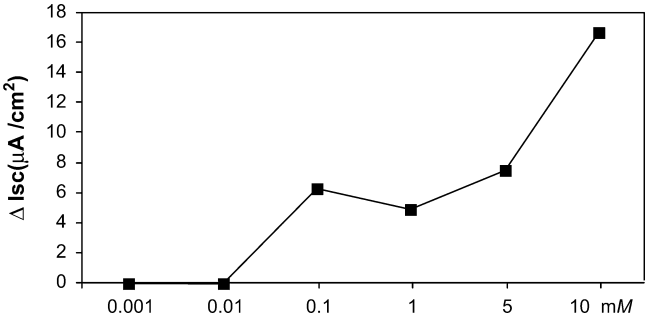


**FIGURE 3.** Ion transport following olsalazine addition.

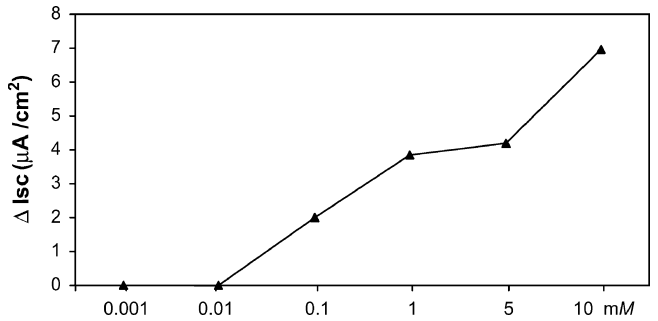


**FIGURE 4.** Ion transport following balsalazide addition to the brush border medium.

inflamed tissue. Isotope flux studies and buffer-free exchange studies indicated that azo compounds stimulated a bicarbonate secretion and NaCl secretion in the ileum without an effect in the colon. In the previous study, intracellular mediators were investigated. The cyclic adenosine monophosphate and cyclic guanosine monophosphate levels did not differ following the response induced by the azo compound. In addition, an inhibitor of cyclooxygenase did not inhibit this secretagogue effect.

The clinically useful doses and associated mucosal concentrations are listed in Table 1. However, when comparing the side effects of pro-drugs, the doses must be compared with the delivered concentrations of 5-ASA (Table 1). For example, some studies[11] have shown that a dose of 6.75 g of balsalazide daily (equal to about 2.4 g of 5-ASA) is sufficient to achieve an optimal response. Yet mesalamine is often administered to patients in doses up to 4.8 g of 5-ASA. Another well-established treatment of ulcerative colitis, sulfasalazine, is composed of 40% 5-ASA, and the usual doses of sulfasalazine are 2 to 4 g per day (equal to 0.8 to 1.6 g of 5-ASA). Olsalazine, a 5-ASA dimer, provides equal doses of 5-ASA but has been shown to induce dose-related diarrhea above a dose of 2 g per day. The side effects of these drugs are often compared throughout the literature based on their optimal dose; however, it is necessary to compare equimolar doses of 5-ASA to these compounds, as observed in the current study. Importantly, if these drugs were to be evaluated at equimolar doses (i.e., the equivalent of a dose of 4.8 g/d of 5-ASA), differences in side effects (such as secretory diarrhea) would likely become even more pronounced.

Clinically, these pro-drugs have been demonstrated to induce side effects leading to increased diarrhea. Balsalazide is effective and better tolerated than sulfasalazine in the short term and for maintenance treatment of ulcerative colitis.[9,17,18] Additionally, bowel frequency improved more rapidly after balsalazide administration compared with that of sulfasalazine after 2 and 4 weeks of treatment, respectively.[19] Similarly, in one study[13] when olsalazine was administered at 1 g per day, approximately 12.5% of patients discontinued therapy due to watery diarrhea, particularly those patients with active colitis. This has limited the potentially higher dosing of olsalazine, as higher doses are more likely to induce symptomatic diarrhea. Finally, 20% to 40% of patients to whom sulfasalazine is administered experience side effects, which is usually attributed to the sulfapyridine moiety.[17] Sulfapyridine-induced intolerance limits the dosing of sulfasalazine to maximally tolerated doses of 4 to 6 g per day, providing the equivalent of 1.6 to 2.4 g of mesalamine, which may mask potential diarrhea from the highest doses that would deliver the equivalent of >2.4 g of 5-ASA. In contrast, mesalamine formulations that are devoid of azo bonds are tolerated without diarrhea or increased side effects at doses up to 4.8 g per day.[20]

There are several clinically relevant aspects to these observations. The first is the limitation of higher dosing with the azo-bond formulations that is related to small bowel secretion, particularly in the setting of active disease. With olsalazine, looser stools often resolve as the colon heals and, presumably, adapts to an increased fluid load.[21] Similar ileal secretion may limit higher dosing for balsalazide and sulfasalazine. It is also possible that clinical efficacy would be decreased with increased diarrhea due to loss of the compound, as has been shown with olsalazine.[2]

Theoretically, the secretory effects could account for the potential benefits, unproven but speculated, of the azo pro-drugs olsalazine and balsalazide for patients with left-sided colitis that have been suggested by post hoc analysis.[22,23] In patients with distal colitis, there often is delayed transit in the right colon,[24–26] and olsalazine has been demonstrated to accelerate transit,[26] potentially delivering more mesalamine to distal sites without prior acetylation, which would impair clinical efficacy.[27]

In conclusion, therapy for the treatment of ulcerative colitis should be based on factors such as efficacy, dose response, and 5-ASA concentrations reaching the epithelium. 5-ASA has been demonstrated to be an effective therapeutic agent by the decrease of intestinal inflammation. This study is the first to report that, with equimolar concentrations of each pro-drug, there is a dose-dependent increase in ileal secretion. This effect was not seen with mesalamine by itself. These findings help to explain the dose limitations of azo-bound pro-drugs in the treatment of inflammatory bowel disease based on their secretory effects within the ileum, which can result in increased diarrhea.

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