INTRODUCTION

Sulfasalazine is the most widely prescribed drug for the treatment of inflammatory bowel disease (1), the long term treatment of ulcerative colitis (2,3) and Crohn’s disease (4). Sulfasalazine is a conjugate of 5-aminosalicylic acid (5-ASA) and sulfapyridine (SP) linked by an azo bond. Following oral administration, sulfasalazine is metabolized by the bacterial azoreductase enzymes in the colon (Fig. 1), reducing the azo bond and releasing these two components (5). Sulfasalazine itself may serve only as a produg to deliver the metabolic products, 5-ASA (a possible anti-inflammatory agent) and SP (an antibacterial agent), to the colon (6-9).

Riboflavin functions metabolically in the form of the two co-enzymes, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) (10). The addition of flavins to in vitro tissue preparations enhanced the activities of the enzymes responsible for degrading azo compounds (11). Further hepatic carcinogenesis by azo dyes is potentiated by riboflavin deficiency (12-18), suggesting that the riboflavin, FAD and FMN affect azo reductase activity in the liver.

Khan et al. (19) reported that the cleavage of sulfasalazine at the azo bond in bacterial suspension and tissue homogenates in vitro was enhanced by NADP+, FAD+, and glucose 6-phosphate, suggesting that azoreduction is NADPH dependent and accelerated by flavins.

Although intestinal bacteria play an important role in the metabolism of sulfasalazine (20), no reports describing the effect of riboflavin in vivo on the reduction of the azo bond in sulfasalazine by the intestinal flora or in the liver have been published. This study reports the effect of oral riboflavin on the urinary excretion and pharmacokinetics of sulfasalazine metabolites in the rat.

MATERIALS AND METHODS

Materials

Sulfasalazine (lot 13F 0731), sulfapyridine (lot 54F 0635), 5-aminosalicylic acid (lot 15F 0803) and riboflavin (lot 16F 0216) were obtained from Sigma Chemical Company (St. Louis, MO). Acetyl-sulfapyridine (Ac-SP) and acetyl-5-aminosalicylic acid (Ac-5-ASA) were received as a gift from Pharmacia Laboratories (Sweden). All other chemicals were reagent grade, were purchased commercially, and were used as received.

Methods

Analytical. Analyses of urine and plasma samples for sulfasalazine metabolites were conducted using a reversed-phase HPLC method with uv detection at 254 nm (21). The assay was linear between 0.5 µg to 25 µg/mL for all sulfasalazine metabolite concentrations.

Animals. Male, CD albino strain rats (Charles River Laboratories, Wilmington, MA) weighing between 200–225 gm were used.

Urinary Excretion Studies. Rats were fasted overnight
and water was allowed, ad libitum. A total of twelve rats were randomly assigned to three groups: A, B, and C (n = 4). Rats in Group A were administered 60 mg/kg of sulfasalazine suspended in aqueous media using 1% tragacanth gum by gastric gavage. Rats in Groups B and C were administered 60 mg/kg, respectively. All rats were housed in metabolism cages, and urine samples were collected at the end of 4, 8, 12, 24, and 48 hours, brought to 5 or 10 ml with distilled water and filtered. Diluted urine samples were frozen (−4°C) and stored in amber bottles in the dark until analyzed.

**Oral Absorption Studies.** One day prior to drug administration, rats were prepared surgically for jugular cannulation as described earlier (22). Food was withdrawn 12 to 16 hours prior to dosing. Sulfasalazine (60 mg/kg) and riboflavin were administered as described above, except that riboflavin doses of 5 mg/kg and 10 mg/kg were used for Groups B and C, respectively. Blood (0.25 mL) was drawn from the jugular vein at 0, 1, 2, 3, 4, 6, 8, 12, and 24 hours in heparinized tubes, and the plasma was harvested following centrifugation. The plasma samples were stored frozen (−4°C) and in the dark until analyzed.

**RESULTS AND DISCUSSION**

Table I shows the percent recovery of the metabolites of sulfasalazine (SS) excreted in the urine over 48 hours in rats. The data presented show that when 10 mg/kg of riboflavin (RF) is concomitantly administered with 60 mg/kg of SS, the recoveries of the metabolites (% dose) were significantly higher (α = 0.01; Scheffe’s test [23]) than either the control group (Group A) or the group receiving 1 mg/kg RF (Group B).

Levy and Jusko (24) have shown that RF absorption takes place mainly from the proximal region of the intestinal tract in man, and its absorption is site specific and saturable. The upper limit of intestinal absorption of RF appears to be about 25 mg in normal subjects (25). The RF dose of 10

<table>
<thead>
<tr>
<th>Compound</th>
<th>Group A (60 mg/kg sulfasalazine) (n = 4)</th>
<th>Group B (60 mg/kg sulfasalazine + 1 mg/kg riboflavin) (n = 4)</th>
<th>Group C (60 mg/kg sulfasalazine + 10 mg/kg riboflavin) (n = 4)</th>
<th>ANOVA (α = 0.01)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-Aminosalicylic acid</td>
<td>4.6 ± 0.19</td>
<td>4.9 ± 0.61</td>
<td>8.2 ± 0.8</td>
<td>C &gt; B &amp; A</td>
</tr>
<tr>
<td>Sulfapyridine</td>
<td>9.7 ± 0.16</td>
<td>7.4 ± 0.67</td>
<td>13.9 ± 1.1</td>
<td>C &gt; B &amp; A</td>
</tr>
<tr>
<td>Acetylsulfapyridine</td>
<td>13.7 ± 0.18</td>
<td>11.9 ± 1.0</td>
<td>30.3 ± 1.9</td>
<td>C &gt; B &amp; A</td>
</tr>
<tr>
<td>Acetylaminosalicylic acid</td>
<td>15.3 ± 0.33</td>
<td>13.7 ± 0.63</td>
<td>35.7 ± 4.5</td>
<td>C &gt; B &amp; A</td>
</tr>
</tbody>
</table>

*Each value represents mean 48 hour recovery (% dose ± S.D.) following concomitant oral administration of sulfasalazine and riboflavin in fasting rats.*