Further Studies on the Hydrolysis of Salicyluric Acid in Intestinal Microorganisms and Prolonged Blood Concentration of Salicylic Acid Following Rectal Administration of Salicyluric Acid in Rabbits

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The blood concentrations of salicyluric acid and salicylic acid following intracecal and rectal administration of salicyluric acid were determined in rabbits. Immediate and very extensive salicylic acid formation in the cecum was found following intracecal administration. After rectal administration, a small amount of salicylic acid was absorbed in intact form. The rest was rapidly hydrolyzed to salicylic acid, which was subsequently absorbed. The blood concentration of salicylic acid was maintained at 1.3—1.8 μg/ml from 2 to 12 h. Three doses of salicyluric acid were administered rectally. The peak level of salicyluric acid increased with dose. However, salicylic acid concentration in the blood following administration of salicyluric acid at 10.0 mg/kg (salicylic acid equivalent) was not double that observed following administration of salicyluric acid at 5.0 mg/kg (salicylic acid equivalent). It appears that a larger amount of salicyluric acid in the rectal lumen may have saturated the glycine deconjugation system.

Keywords — salicyluric acid; salicylic acid; gut flora; microorganism; presystemic deconjugation; glycine conjugate; cecal administration; rectal administration; prodrug; prolonged blood concentration

Introduction

The significance of the gut flora to biopharmacy has been emphasized with respect to their ability to metabolize drugs within the intestinal lumen. The hydrolysis of glycine conjugates by gastrointestinal microorganisms is well documented. Isonicotinuric acid, salicyluric acid, p-aminohippuric acid, and p-acetylaminohippuric acid appear to be hydrolyzed by gastrointestinal bacteria following oral administration to man.

In the previous report, we demonstrated that salicyluric acid is metabolized to salicylic acid by gut flora in rabbits. Salicylic acid and unchanged salicyluric acid were detected in the blood after oral administration of salicyluric acid. Furthermore, the contents from the jejunum and the ileum lacked salicylic acid hydrolyzing activity, whereas the contents from the cecum and colon were the major source of metabolism.

In order to investigate the possible mechanism of hydrolysis of salicyluric acid in the intestinal microorganisms, we examined the intracecal and rectal administration of salicyluric acid in rabbits.

Materials and Methods

Materials — Salicyluric acid was obtained from Sigma Chemical Co. (St. Louis, U.S.A.). Sodium salicylate and acetonitrile were purchased from Nakarai Chemicals Ltd. (Kyoto, Japan). o-Methoxybenzoic acid was of reagent grade. All other chemicals used in these experiments were of the finest grade available.

Animal Experiments — Male albino rabbits weighing 1.8—2.5 kg were used throughout the study. The animals were housed in an air-conditioned room and maintained on a standard laboratory diet (ORC4, Oriental Yeast Co., Ltd., Tokyo, Japan). The rabbits were starved for about 20 h prior to use for experiments but had free access to water. Sodium salicylate was dissolved in distilled water and salicyluric acid in NaOH (equivalent to salicylic acid). Appropriate amounts of drug solution were administered
intracereally and rectally.

Intracecal Administration of Drug: Animals were anesthetized with pentobarbital, given intravenously, via ear vein. After complete anesthesia, a midline incision (2–3 cm) was made, and the drug solution (6 ml/kg) was administered by direct injection into the cecum by syringe. Leakage of drug solution at the injection site was not observed. The abdomen was closed with operative stitching.

Rectal Administration of Drug: The drug solution (2 ml/kg) was administered rectally, and the anus was closed with a plastic clip to prevent leakage of the rectal contents during the experiments. Following intracecal or rectal administration of drug, blood was collected with a heparinized syringe at appropriate time intervals from an ear vein.

**Analytical Method** — Salicyluric acid and salicylic acid in blood were analyzed by high-performance liquid chromatography after modifying the method described by Cham *et al.* We used fluorescence intensity for detection instead of absorption measurement at 313 nm, which was employed by Cham *et al.* Blood samples (0.4 ml) were added to an equal volume of acetonitrile containing 30 μg of the internal standard, o-methoxybenzoic acid, in 1 ml. The samples were mixed on a vortex-type mixer and centrifuged at 10000 rpm for 10 min. Then 20 μl of the supernatant fluid was withdrawn using a Hamilton syringe and loaded onto the column. Calibration curves were constructed from data on the peak-height ratios of salicyluric acid and salicylic acid to the internal standard. We used a Trirotar-II pump, an FP-110 fluorescence detector, and an RC-125 recorder (all from Japan Spectroscopic Co., Ltd., Tokyo, Japan). The prepared column was a bonded octadecylsilane-silica gel type (Fine SIL C 18, Japan Spectroscopic Co., Ltd.), average particle size 10 μm and 250 × 4.6 mm internal dimensions. This column was used at room temperature. The peak-height of fluorescence intensity was recorded at excitation and emission wavelengths of 300 and 410 nm, respectively. The chromatographic mobile phase consisted of a mixture of acetic acid–methanol–water (4:40:60, v/v/v) and was filtered by passing through a 0.45 μm pore size membrane filter (Toyo Roshi Co., Ltd., Tokyo, Japan) before use. The flow rate was 1.5 ml/min. The retention times of salicyluric acid, salicylic acid and the internal standard were 4.9, 9.5 and 7.5 min, respectively.

**Results and Discussion**

Boxenbaum *et al.* demonstrated that salicyluric acid undergoes intestinal microbial metabolism to salicylic acid prior to absorption in healthy subjects. Following the oral administration of salicyluric acid at 3 mg/kg, approximately 80% of the salicyluric acid dose was absorbed intact from the upper gastrointestinal tract; the remaining salicyluric acid apparently passed down to the large intestine and was subjected to microbial hydrolysis to salicylic acid. In the previous report, we examined the fate of salicyluric acid after oral administration in rabbits. Salicylic acid and unchanged salicyluric acid were detected in the blood. Following intravenous administration of salicyluric acid, only unchanged salicyluric acid was detected in the blood, suggesting that presystemic deconjugation of glycine was involved. After treatment of rabbits with kanamycin sulfate, complete inhibition of the formation of salicylic acid following oral administration of salicyluric acid was demonstrated, indicating that the intestinal microflora were responsible for the biotransformation. Furthermore, *in vitro* incubation of salicyluric acid with gut contents showed that the major location of the hydrolysis was the hind gut.

Scheline reviewed much of the literature on the distribution of microorganisms in the gastrointestinal tract and indicated that the stomach, duodenum, jejunum and upper ileum are only sparsely populated. Increasing numbers of organisms exist in the distal ileum, and a significant increase is seen at the ileocecal valve in humans.

In order to examine the mechanism of hydrolysis of salicyluric acid in the intestinal microorganisms in rabbits, salicyluric acid was administered intracereally. As shown in Fig. 1, salicylic acid reached a peak blood concentration in about 2 h, after which it slowly declined.
Salicyluric Acid Hydrolysis in Rabbits

Fig. 1. Blood Concentration of Salicyluric Acid and Salicylic Acid Following Intracecal Administration of Salicyluric Acid

○, salicyluric acid; ●, salicylic acid. Blood concentration and dose (5.0 mg/kg) of salicyluric acid: salicylic acid equivalent. Each point represents the mean ± S.E. of five experiments.

Fig. 2. Blood Concentration of Salicylic Acid Following Intracecal Administration of Salicylic Acid

Dose: 5.0 mg/kg of salicylic acid. Each point represents the mean ± S.E. of five experiments.

Fig. 3. Blood Concentration of Salicyluric Acid and Salicylic Acid Following Rectal Administration of Salicyluric Acid

○, salicyluric acid; ●, salicylic acid. Blood concentration and dose (5.0 mg/kg) of salicyluric acid: salicylic acid equivalent. Each point represents the mean ± S.E. of four experiments.

ministration of salicyluric acid in rabbits, salicylic acid was detected in the blood at 2 h and reached the maximum level at 5 h.\(^7\) From these results, it seems that the delays in detection and the maximum levels of salicylic acid following oral administration of salicyluric acid are mainly due to the time required for the gastrointestinal transit and partially due to the hydrolysis to salicylic acid.

The rectal route has a definite advantage over the oral route for drugs that are destroyed by gastric acidity or by enzymes in the intestinal wall. Potentially, the rectal route may also partially reduce first-pass hepatic loss. The previous report\(^7\) showed that the luminal contents of the colon and feces were responsible for salicyluric acid hydrolysis. These results suggest that
TABLE I. Blood Concentration of Salicyluric Acid and Salicylic Acid Following Rectal Administration of Three Doses of Salicyluric Acid

<table>
<thead>
<tr>
<th>Time</th>
<th>SU (2.5 mg/kg, N = 4)</th>
<th>SA (2.5 mg/kg, N = 4)</th>
<th>SU (5.0 mg/kg, N = 4)</th>
<th>SA (5.0 mg/kg, N = 4)</th>
<th>SU (10.0 mg/kg, N = 7)</th>
<th>SA (10.0 mg/kg, N = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 min</td>
<td>0.18 ± 0.12</td>
<td>0.11 ± 0.07</td>
<td>0.33 ± 0.14</td>
<td>0.36 ± 0.14</td>
<td>1.89 ± 0.49</td>
<td>1.02 ± 0.20</td>
</tr>
<tr>
<td>15 min</td>
<td>0.14 ± 0.13</td>
<td>0.24 ± 0.09</td>
<td>0.35 ± 0.15</td>
<td>0.45 ± 0.14</td>
<td>1.39 ± 0.37</td>
<td>0.85 ± 0.12</td>
</tr>
<tr>
<td>30 min</td>
<td>0.13 ± 0.12</td>
<td>0.29 ± 0.08</td>
<td>0.16 ± 0.10</td>
<td>0.58 ± 0.21</td>
<td>0.82 ± 0.24</td>
<td>0.85 ± 0.11</td>
</tr>
<tr>
<td>1 h</td>
<td>0.05 ± 0.05</td>
<td>0.45 ± 0.18</td>
<td>0.17 ± 0.07</td>
<td>0.80 ± 0.21</td>
<td>0.56 ± 0.13</td>
<td>0.97 ± 0.11</td>
</tr>
<tr>
<td>1.5 h</td>
<td>0.05 ± 0.05</td>
<td>0.48 ± 0.20</td>
<td>0.09 ± 0.04</td>
<td>0.86 ± 0.22</td>
<td>0.37 ± 0.10</td>
<td>1.13 ± 0.11</td>
</tr>
<tr>
<td>2 h</td>
<td>0.30 ± 0.06</td>
<td>0.09 ± 0.06</td>
<td>1.34 ± 0.32</td>
<td>0.29 ± 0.10</td>
<td>1.21 ± 0.17</td>
<td>0.62 ± 0.29</td>
</tr>
<tr>
<td>3 h</td>
<td>0.04 ± 0.14</td>
<td>1.38 ± 0.30</td>
<td>0.16 ± 0.03</td>
<td>1.42 ± 0.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 h</td>
<td>0.74 ± 0.18</td>
<td>0.02 ± 0.01</td>
<td>1.49 ± 0.28</td>
<td>0.05 ± 0.03</td>
<td>1.63 ± 0.27</td>
<td></td>
</tr>
<tr>
<td>5 h</td>
<td>0.81 ± 0.28</td>
<td>0</td>
<td>1.45 ± 0.29</td>
<td>0.06 ± 0.06</td>
<td>1.84 ± 0.31</td>
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</tr>
<tr>
<td>6 h</td>
<td>0.87 ± 0.30</td>
<td>0.51 ± 0.19</td>
<td>0.02 ± 0.02</td>
<td></td>
<td>2.18 ± 0.38</td>
<td></td>
</tr>
<tr>
<td>8 h</td>
<td>0.93 ± 0.30</td>
<td>1.87 ± 0.23</td>
<td>0</td>
<td></td>
<td>2.67 ± 0.46</td>
<td></td>
</tr>
</tbody>
</table>

SU: salicyluric acid. SA: salicylic acid. N: number of animal experiments. Each value represents the mean ± S.E.

The blood concentration and dose of salicyluric acid: salicylic acid equivalent. Salicylic acid was hydrolyzed by microorganisms within the rectum and that liberated salicylic acid is subsequently absorbed. Figure 3 shows the blood concentration of salicyluric acid and salicylic acid following rectal administration of salicyluric acid (5.0 mg/kg; salicylic acid equivalent). A small amount of salicyluric acid was absorbed in intact form. The rest was rapidly hydrolyzed to salicylic acid, which was subsequently absorbed. The blood concentration of salicylic acid was maintained at 1.3–1.8 µg/ml from 2 to 12 h. The blood concentration of salicylic acid following rectal administration of salicyluric acid is shown in Fig. 4. Salicylic acid reached a peak blood concentration in about 30 min, suggesting rapid absorption from the rectum. These results indicate that microbial metabolism of salicyluric acid may be responsible for the prolonged retention of salicylic acid in the blood.

Three doses of salicyluric acid were administered rectally; the results are presented in Table I. The peak level of salicyluric acid increased with dose. Following administration of 2.5 and 5.0 mg/kg salicyluric acid (salicylic acid equivalent), a linear tendency was found in the blood concentration of salicylic acid. However, salicylic acid concentration in the blood following administration of salicyluric acid at 10.0 mg/kg (salicylic acid equivalent) was not double that observed following administration of salicyluric acid at 5.0 mg/kg (salicylic acid equivalent). It appears that a larger amount of salicylic acid in the rectal lumen may have saturated the glycine deconjugation system.

Prodrugs have been used in drug delivery systems. A prodrug is inactive as administered, but its physicochemical properties permit its activation in vivo once it reaches its target. In these cases, the prodrug is converted to the parent drug chemically or by specific enzymes at the target site. Salicylasulapyridine (sulfasalazine) has long been a widely used drug for the treatment of ulcerative colitis. When sulfasalazine after oral administration reaches the colon, the azo bond is split by azo-reductases from colonic bacteria, resulting in the formation of 5-aminosalicylic acid and sulfapyridine. Recently, Friend and Chang reported that steroid glycosides and the unique glycosidase activity of the colonic microflora form the basis of a colon-specific drug delivery system. Drug glycosides are hydrophilic and, thus, are poorly absorbed from the small intestine. Once such a glycoside reaches the colon it can be cleaved by bacterial glycosidases, releasing the free drug to be absorbed by the colonic mucosa.
The present results suggest the importance of rectal administration of the prodrug, which is converted to the parent drug by microorganisms, to obtain the prolonged blood concentration of the parent drug. There may be differences in the metabolic fate of salicyluric acid among different species. Therefore, additional studies are needed in other animal species.

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References