Effect of Oral Pretreatment with Indomethacin on the Intestinal First-Pass Metabolism of Salicylamide in Rabbits

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(Received March 22, 1988)

The effect of oral pretreatment with indomethacin on the intestinal first-pass metabolism of salicylamide (SAM) was studied in rabbits using in situ intestinal sacs with complete mesenteric venous blood collection. The appearance of both SAM and its metabolites into the mesenteric venous blood was measured directly by cannulating the mesenteric vein of exposed rabbit intestine and collecting all venous blood draining from the absorbing region. By oral pretreatment with indomethacin, the total amounts of SAM absorbed in 20 and 120 min were significantly increased compared to the control. These results indicated the alteration of the permeability in the intestinal mucosa. In 20 min, indomethacin pretreatment resulted in increased appearance of SAM and SAM glucuronide in the mesenteric venous blood. In 120 min, increased appearance of SAM and decreased appearance of SAM sulfate were observed, compared to the control. These findings suggested that the change in the intestinal first-pass metabolism of SAM is probably due to the intestinal mucosal damage by oral pretreatment with indomethacin.

Keywords — indomethacin; first-pass metabolism; first-pass effect; salicylamide; membrane permeability; intestinal absorption; intestinal metabolism; membrane transport; mucosal damage; rabbit

Introduction

Salicylamide (SAM), a mild analgesic and antipyretic agent, was reported to exhibit first-pass metabolism during intestinal absorption in rabbits,1–4) rats3,5–7) and dogs.8,9) SAM is metabolized to SAM glucuronide (SAMG) and SAM sulfate (SAMS) in the intestine. The study of the first-pass metabolism of drug in the intestine was usually carried out in normal animals. We postulated that the intestinal metabolism of drug would be influenced in mucosa with damage. It is known that administration of large doses of steroidal as well as non-steroidal anti-inflammatory agents including indomethacin causes gastrointestinal mucosal damage. In previous reports,10–13) we examined the permeation of phenolsulphonphthalein, a poorly absorbed drug, as an index of an assessment of gastrointestinal mucosal damage in vivo. The urinary recovery after oral administration of phenolsulphonphthalein was significantly increased in rats with indomethacin (100 mg/kg)-induced mucosal damage.10,11) However, little attention has been paid to the assessment of the mucosal damage by the metabolism of marker compound in the intestine. The present study was undertaken to investigate the intestinal first-pass metabolism of SAM in rabbits pretreated with indomethacin orally.

Materials and Methods

Materials — SAM was obtained from Nakalai Tesque, Inc. (Kyoto, Japan), indomethacin from Sigma Chemical Co. (St. Louis, MO., U.S.A.), carboxymethyl cellulose sodium salt (CMC) from Hayashi Pure Chemical Industries, Ltd. (Osaka, Japan), β-glucuronidase from Tokyo Zohki Kagaku Co., Ltd. (Tokyo, Japan) and β-glucuronidase/aryl sulfatase from Boehringer Mannheim GmbH (Mannheim, Federal Republic of Germany). All other reagents used in these experiments were of the finest grade available.

Animal Experiments — Male albino rabbits, weighing 2.0–2.6 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). Indomethacin (500 mg/kg) suspended in 1% CMC solution was administered by gastric intubation. The rabbits were starved for 24 h prior to the absorption ex-
periments but had free access to water. The control rabbits did not receive the CMC solution.

In situ rabbit intestinal sacs with complete mesenteric venous blood collection were prepared as reported by Barr and Riegelman.\textsuperscript{1} The technique of collecting all venous blood draining from the region of absorption was developed to provide an in vivo preparation with intact circulation. The usefulness of this preparation is that free drug and drug metabolites which are absorbed into the capillary blood can be collected in the venous effluent and not reach general circulation. Animals were anesthetized with pentobarbital, given intravenously, via an ear vein. Additional pentobarbital was administered as necessary during the experiment to maintain anesthesia. After complete anesthesia, a midline incision was made, and the mid-ileal portion of the intestine (5–8 cm) was cut. The intestinal lumen was washed with 0.9% NaCl, and both sides of the mid-ileal portion of intestine were ligated to prepare a closed sac. This portion was selected because of its accessibility and suitable vasculature to facilitate cannulation. The mesenteric vein was cannulated with a polyethylene tubing (SP 45, i.d. 0.58 mm, o.d. 0.96 mm, Natsume Seisakusho Co., Ltd., Tokyo, Japan). The coagulation of blood was prevented by the intravenous administration of heparin (1000 I.U.). All venous blood was collected in centrifuge tubes and assayed for SAM and its metabolites. The amounts of SAM and its metabolites in the blood were estimated by multiplying the volume of blood collected by the concentration of SAM and its metabolites in the blood. The drug solution (3 ml) was administered by direct injection into the intestinal sac by syringe. The drug was dissolved in the pH 7.2 buffer solution reported by Schanker and Tocco.\textsuperscript{14} The blood lost from the mesenteric vein was replaced continuously by an intravenous infusion of 0.9% NaCl via the ear vein. The isolated intestine was kept warm by a lamp and moist by frequent application of 0.9% NaCl to a paper covering the intestine. Determination of the appearance of SAM and its metabolites in the intestinal luminal solution was carried out as follows. At the end of an absorption period, the intestinal closed sac was isolated by tearing off the mesentery and the serosal surface was blotted by paper. The intestinal luminal solution was withdrawn as completely as possible, and the intestinal lumen was washed with distilled water. The washings were combined with the intestinal luminal solution and made up to 100 ml with distilled water. This sample solution was assayed for SAM and its metabolites. Results were compared statistically using Student’s t-test.

**Analytical Methods** — SAM, SAMG and SAMS were quantitiated from venous blood and intestinal luminal solution by the spectrofluorometric assay method described in a previous report.\textsuperscript{3} A Shimadzu RF-510 spectrofluorometer (Shimadzu Co., Ltd., Kyoto, Japan) was used. Glucuronide and sulfate were analyzed after the hydrolysis of the sample with β-glucuronidase or β-glucuronidase/arylsulfatase at 37 °C for 24 h.

**Results and Discussion**

The effect of oral pretreatment with indomethacin on the intestinal first-pass metabolism of SAM was examined in rabbits using in situ intestinal sacs with complete mesenteric venous blood collection. The appearance of both SAM and its metabolites into the mesenteric venous

![Graph](image-url)
blood was measured directly by cannulating the mesenteric vein of exposed rabbit intestine and collecting all venous blood draining from the absorbing region. SAM is metabolized to SAMG and SAMS in the rabbit intestine. Minor metabolites of SAM were not determined in this study.

Figures 1, 2 and 3 show the appearance of SAM, SAMG and SAMS, respectively in the mesenteric venous blood after an injection of SAM into the intestinal lumen. As shown in Fig. 1, indomethacin pretreatment resulted in enhanced SAM appearance compared to the control. The cumulative amounts of SAM in the mesenteric venous blood tended to reach a plateau in 30 min, suggesting the rapid absorption of SAM in the intestine. Figure 2 shows the appearance of SAMG in the mesenteric venous blood. Following oral pretreatment with indomethacin, the appearance of SAMG increased at the beginning of the absorption period compared to the control. However, no effect was found in cumulative amounts of SAMG in 120 min. As shown in Fig. 3, indomethacin pretreatment resulted in a decreased appearance of SAMS in 120 min compared to the control. However, no effect was observed in the appearance of SAMS in the mesenteric venous blood at the beginning of the absorption period. The results of appearance of SAM and its metabolites in 20 and 120 min in the mesenteric venous blood after an injection of SAM are summarized in Table I.

Table I. Appearance of SAM and Its Metabolites in the Mesenteric Venous Blood after an Injection of SAM

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time (min)</th>
<th>Total amount absorbed (% of dose)</th>
<th>SAM (Cumulative appearance amounts, μg)</th>
<th>SAMG (Cumulative appearance amounts, μg)</th>
<th>SAMS (Cumulative appearance amounts, μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20</td>
<td>30.1 ± 9.2 (5)</td>
<td>17.0 ± 11.2</td>
<td>33.2 ± 10.4</td>
<td>10.0 ± 4.5</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>20</td>
<td>59.0 ± 10.6α (4)</td>
<td>45.2 ± 5.5α</td>
<td>61.0 ± 13.5α</td>
<td>11.6 ± 3.5</td>
</tr>
<tr>
<td>Control</td>
<td>120</td>
<td>62.8 ± 6.0 (5)</td>
<td>19.2 ± 12.3</td>
<td>78.1 ± 6.9</td>
<td>28.3 ± 7.7</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>120</td>
<td>74.1 ± 4.9β (4)</td>
<td>45.8 ± 4.5β</td>
<td>85.0 ± 6.5</td>
<td>17.3 ± 3.5β</td>
</tr>
</tbody>
</table>

Dose: 3 ml of 67 μg/ml solution of SAM. Each value is expressed as the mean ± S.D. Numbers in parentheses represent number of experiments. Statistical significance: α) p < 0.01, β) p < 0.02, γ) p < 0.05. The amounts of SAMG and SAMS were calculated as SAM. Total amount absorbed represents the sum of SAM, SAMG and SAMS.
TABLE II. Appearance of SAM and Its Metabolites in the Intestinal Luminal Solution

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time (min)</th>
<th>Total amount appeared (% of dose)</th>
<th>SAM (Appearance amounts, μg)</th>
<th>SAMG</th>
<th>SAMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>120</td>
<td>20.7 ± 4.4 (5)</td>
<td>1.1 ± 1.4</td>
<td>23.3 ± 3.9</td>
<td>16.9 ± 3.9</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>120</td>
<td>16.9 ± 5.8 (4)</td>
<td>0</td>
<td>27.8 ± 10.0</td>
<td>6.0 ± 2.8a</td>
</tr>
</tbody>
</table>

Dose: 3 ml of 67 μg/ml solution of SAM. Each value is expressed as the mean ± S.D. Numbers in parentheses represent number of experiments. Statistical significance: a) p < 0.01. The amounts of SAMG and SAMS were calculated as SAM. Total amount appeared represents the sum of SAM, SAMG and SAMS.

min were significantly increased compared to the control. These results indicated the alteration of the permeability in the intestinal mucosa. It is well known that indomethacin gives rise to a high incidence of gastrointestinal side effects. Anorexia, nausea, vomiting, epigastric burning or abdominal pain, and diarrhea have been reported in patients receiving indomethacin. Somogyi et al., Kent et al., and Brodie et al. reported that administration of indomethacin caused extensive lesions in the lower small intestine in rats, characterized by peritonitis, ulceration and occasional frank necrosis. In the present study, microscopical examination of intestinal mucosa was not performed. As shown in Table I, the appearance of SAM and its metabolites in the mesenteric venous blood was affected following oral pretreatment with indomethacin.

To provide more information on intestinal first-pass metabolism of SAM, the appearance of SAM and its metabolites in the intestinal luminal solution after injection of SAM was examined. The results are presented in Table II. In control rabbits, 1.1 μg of SAM (0.5% of dose) appeared in the intestinal luminal solution. SAM was not detected in the indomethacin-pretreated rabbits. Both control and indomethacin-pretreated rabbits showed almost complete absorption of SAM. A significant decrease of SAMS appearance in the intestinal luminal solution was observed in rabbits pretreated with indomethacin. No effect was found in the appearance of SAMG in the intestinal luminal solution. These results were reflected in the appearance of SAMG and SAMS in the mesenteric venous blood in 120 min.

In the present study, we did not examine the effect of indomethacin on drug-metabolizing enzymes in the intestine. Falzon et al. reported that intraperitoneal administration of indomethacin to rats caused a significant decrease in hepatic glucuronyl transferase activity. Sulphotransferase was not affected by indomethacin.

From the results described above, it is suggested that the change in intestinal first-pass metabolism of SAM is probably due to intestinal mucosal damage by oral pretreatment with indomethacin. It is known that intestinal metabolism plays a role in determining the bioavailability of orally administered drugs. If the absorption and first-pass metabolism of drugs change in the intestine with mucosal damage, dosage adjustment may be required.

The damaging effects of drugs on the gastrointestinal tract are considered in terms of the gastrointestinal mucosal barrier, gastrointestinal erosions and microbleeding. It is usually assessed by macroscopical and microscopical examination. Measurement of gastrointestinal blood loss is also used extensively. The alteration of first-pass metabolism of marker compounds may be utilized for an assessment of intestinal mucosal damage.

Acknowledgement We wish to thank Michihiro Katayama, Masae Kikuchi and Kazuko Kunisawa for skilled technical assistance.

References


