# An Assessment of Mucosal Damage *in Vivo*: Effect of Oral Pretreatment with 5-Fluorouracil on the Intestinal First-Pass Metabolism of Salicylamide in Rabbits

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**An assessment of 5-fluorouracil (5-FU)-induced mucosal damage *in vivo* by measuring the metabolism of salicylamide (SAM) was investigated in rabbit intestine. The mucosal damage in the intestine 48 h after oral administration of 5-FU (30 mg/kg) was examined using a scanning electron microscope. By the oral pretreatment with 5-FU, the morphological changes of jejunal and ileal mucosa were recognized compared with the control. The intestinal first-pass metabolism of SAM was studied 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 intestine and collecting all venous blood draining from the absorbing region. Following oral pretreatment with 5-FU, the appearance of SAM glucuronide (SAMG) in the mesenteric venous blood was significantly increased. The increased blood concentration of SAMG following intraduodenal administration of SAM *in vivo* was observed in rabbits pretreated with 5-FU orally. However, the blood concentration of SAMG after intravenous administration of SAM was not increased compared with the control. These findings suggest that the change in intestinal first-pass metabolism of SAM may be due to the intestinal mucosal damage by oral pretreatment with 5-FU. The alteration of intestinal first-pass metabolism of a marker compound may be utilized for the assessment of intestinal mucosal damage *in vivo*.**

**Keywords** mucosal damage; intestinal metabolism; 5-fluorouracil; salicylamide; rabbit; intestinal absorption; screening test; membrane permeability; membrane transport; assessment

The damaging effects of drugs on the gastrointestinal tract are considered in terms of the gastrointestinal mucosal barrier, gastrointestinal erosions and microbleeding and whether peptic ulcers are caused. It is usually assessed by macroscopical and microscopical examination. Measurement of gastrointestinal blood loss is also used extensively. A few studies have investigated an assessment of the gastrointestinal mucosal damage using the permeability of marker compounds in experimental animals and humans. An attempt has been made to examine the intestinal mucosa in celiac sprue by permeability of low molecular weight polyethylene glycols in humans.[1,2] Also, Cobden *et al.* reported a change in intestinal permeability of cellobiose and mannitol in celiac disease in humans.[3,4] Furthermore, intestinal permeability was assessed in patients with inflammatory bowel disease by measuring the urine excretion of $^{51}$chromium-labeled ethylenediaminetetraacetate after oral administration.[5] In previous reports,[6-9] we examined the permeation of phenolsulfonphthalein, a poorly absorbed drug, as an index of the assessment of gastrointestinal mucosal damage *in vivo*. The urinary recovery after oral administration of phenolsulfonphthalein was significantly increased in rats with indomethacin-induced mucosal damage. However, little attention has been paid to the assessment of the mucosal damage by the metabolism of the marker compound in the intestine. In the present study, an assessment of mucosal damage induced by oral pretreatment with 5-fluorouracil (5-FU) was examined by measuring the metabolism of salicylamide (SAM) as a marker compound in rabbit intestine *in vivo*.

## Experimental

**Materials** 5-FU, SAM and glutaraldehyde (25% in water) were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Carboxymethylcellulose sodium salt (CMC) was obtained from Hayashi Pure Chemical Industries, Ltd. (Osaka, Japan), β-glucuronidase from Tokyo Zohki Kagaku Co., Ltd. (Tokyo, Japan), β-glucuronidase/arylsulfatase from Boehringer Mannheim GmbH (Mannheim, Germany) and heparin sodium salt from Novo Industries, Ltd. (Denmark). All other chemicals used in these experiments were of an analytical or reagent grade.

**Animals** Male albino rabbits obtained from Kyudo Co., Ltd. (Kumamoto, Japan), weighing 2—3 kg, were used throughout the study. The animals were individually housed in cages in an air-conditioned room and maintained on a standard laboratory diet (ORC4, Oriental Yeast Co., Ltd., Tokyo, Japan). 5-FU (30 mg/kg) suspended in 1% CMC solution was administered by gastric intubation. Forty-eight hours after oral administration of 5-FU, a scanning electron micrography of the intestinal mucosa in at least 2 rabbits was carried out. Twenty-four hours after oral administration of 5-FU, rabbits were starved for 24 h. Then, *in situ* and *in vivo* absorption experiments were carried out. During fasting, animals were allowed free access to water only.

**Scanning Electron Micrography of Intestinal Mucosa** The intestinal tract of at least 2 rabbits was removed under anesthesia with sodium pentobarbital (25 mg/kg), given intravenously *via* an ear vein. Two or 3 specimens of intestinal mucosa were placed in a 1% glutaraldehyde solution diluted with a pH 7.3 phosphate buffer solution to fix over 1 h at 4 °C. Mucosal damage in the intestine was observed using a scanning electron microscope (model WS-250, Akashi Beam Technology Co., Tokyo, Japan).

***In Situ* Absorption Experiments** *In situ* rabbit intestinal sacs with complete mesenteric venous blood collection were prepared as reported by Barr and Riegelman.[10] 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 a free drug and its metabolites which are absorbed into the capillary blood can be collected in the venous effluent and not reach the general circulation. Animals were anesthetized with sodium pentobarbital (25 mg/kg), given intravenously *via* an ear vein. Additional sodium pentobarbital was administered as necessary during the experiments to maintain anesthesia. After complete anesthesia, a midline incision was made, and the mid-ileal portion of the intestine (5 cm) was cut. The intestinal lumen was washed with 0.9% NaCl, and both ends of the mid-ileal portion of the 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 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 sodium salt (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.[11] The

blood lost from the mesenteric vein was replaced continuously by an intravenous infusion of 0.9% NaCl *via* an 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 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 the Student's *t*-test.

**Intravenous Administration of SAM** SAM solution (30 mg/kg) dissolved in 0.1 N NaOH was administered intravenously *via* an ear vein. The blood was collected from the vein of another ear with a heparinized syringe at appropriate time intervals.

**Intraduodenal Administration of SAM** Animals were anesthetized with sodium pentobarbital (25 mg/kg), given intravenously *via* an ear vein. After complete anesthesia, a midline incision (5 cm) was made, and SAM solution (30 mg/kg) dissolved in 0.1 N NaOH was administered by direct injection into the duodenum with syringe. Leakage of SAM solution at the injection site was not observed. The blood was collected from an ear vein with a heparinized syringe at appropriate time intervals.

**Analytical Methods** SAM, SAM glucuronide (SAMG) and SAM sulfate (SAMS) were quantitated from venous blood and intestinal luminal solution by the spectrofluorometric assay method reported by Shibasaki *et al.*[12] A Shimadzu RF-510 spectrofluorometer (Shimadzu Co., Ltd., Kyoto, Japan) was used. SAMG and SAMS were analyzed as SAM after the hydrolysis of the sample with β-glucuronidase or β-glucuronidase/arylsulfatase at 37 °C for 24 h.

## Results and Discussion

Chemotherapy with antineoplastic agents is often restricted by toxic side effects, especially those affecting the rapid proliferating tissues including the gastrointestinal tract. 5-FU is a typical pyrimidine analog and has been used clinically for the treatment of carcinoma of the breast and gastrointestinal tract. Schanker *et al.*,[11,13-15] Muranishi *et al.*[16] and Sasaki *et al.*[17] reported the absorption mechanism of this drug in the small intestine of the rat and showed that it crossed the small intestinal epithelium by active transport as well as passive diffusion. Active transport is the predominant mode of absorption at low concentrations, whereas passive diffusion predominates at high concentrations when the active transport process is saturated. It is well known that the earliest untoward symptoms during a course of 5-FU therapy are anorexia and nausea. These are followed by stomatitis and diarrhea. Mucosal ulcerations may occur throughout the gastrointestinal tract. Roche *et al.* demonstrated that the intraperitoneal injection of a single dose of 5-FU in rats caused morphological changes of the intestinal mucosa and a reduction in its ability to absorb glucose.[18] Also, Mizuno *et al.* reported that dosing with 5-FU *via* oral or intravenous routes caused the suppression of L-tryptophan and sulfanilamide absorption in the rat small intestine.[19] Furthermore, Mizuno *et al.*[20-23] and Hamaura *et al.*[24] investigated in detail the characterization of mitomycin C-induced gastrointestinal mucosal damage in rats.

Figure 1 shows scanning electron micrographs of the intestinal mucosa in control rabbits. As shown in Fig. 1A, the duodenal villi were broad and occasionally folded on the longitudinal axis. Individual cells could not be discriminated at this magnification. Tongue-shaped villi were found in the jejunum (Fig. 1B). In Fig. 1C, the ileal villi were broad, tongue-shaped structures. Figure 2 shows scanning electron micrographs of the intestinal mucosa in rabbits 48 h after oral administration of 5-FU. Following the oral pretreatment with 5-FU, the surface character of the duodenal mucosa was almost identical compared with the control (Fig. 2A). In Fig. 2B, however, the villi of the jejunal mucosa were broken down and the villus surface was covered with mucus. As shown in Fig. 2C, a

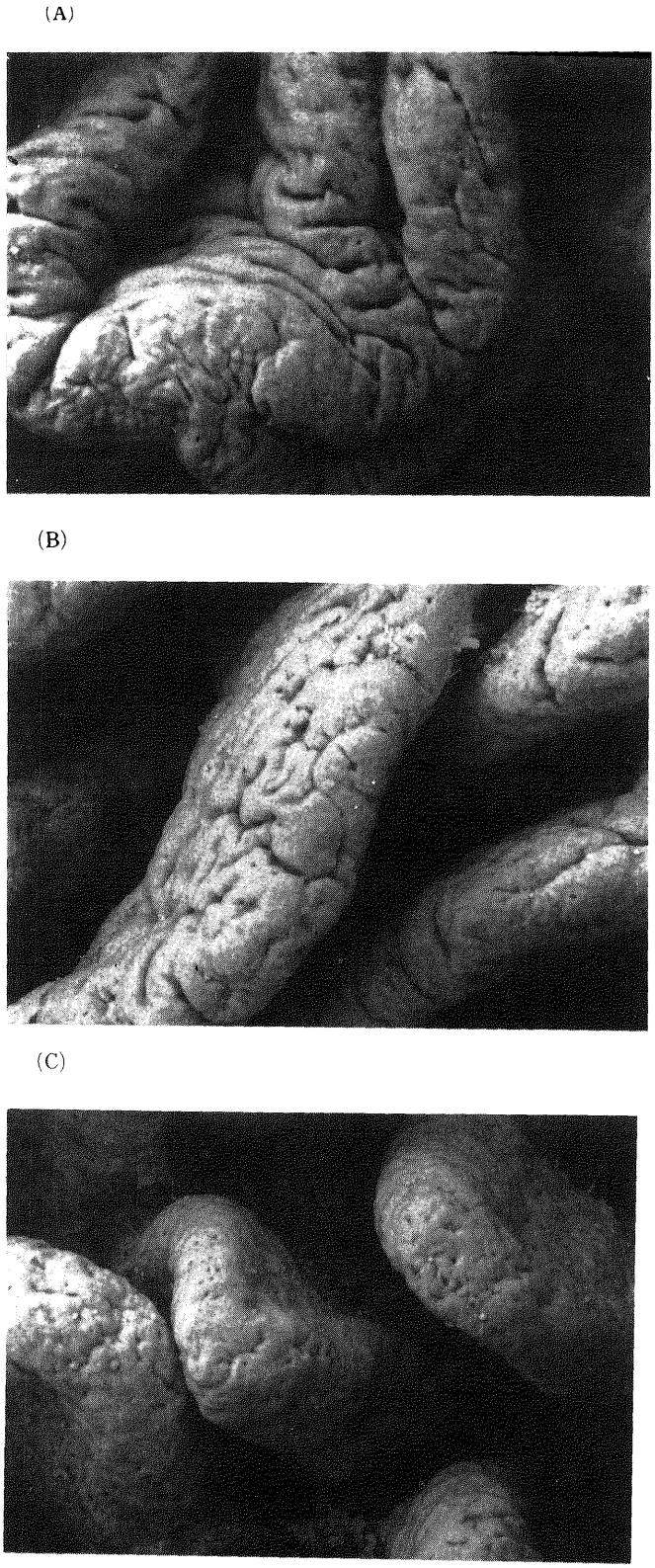


Fig. 1. Scanning Electron Micrographs of Intestinal Mucosa in Control Rabbits

(A) duodenum, (B) jejunum, (C) ileum. Magnification: ×230. These micrographs were reported previously.[38]

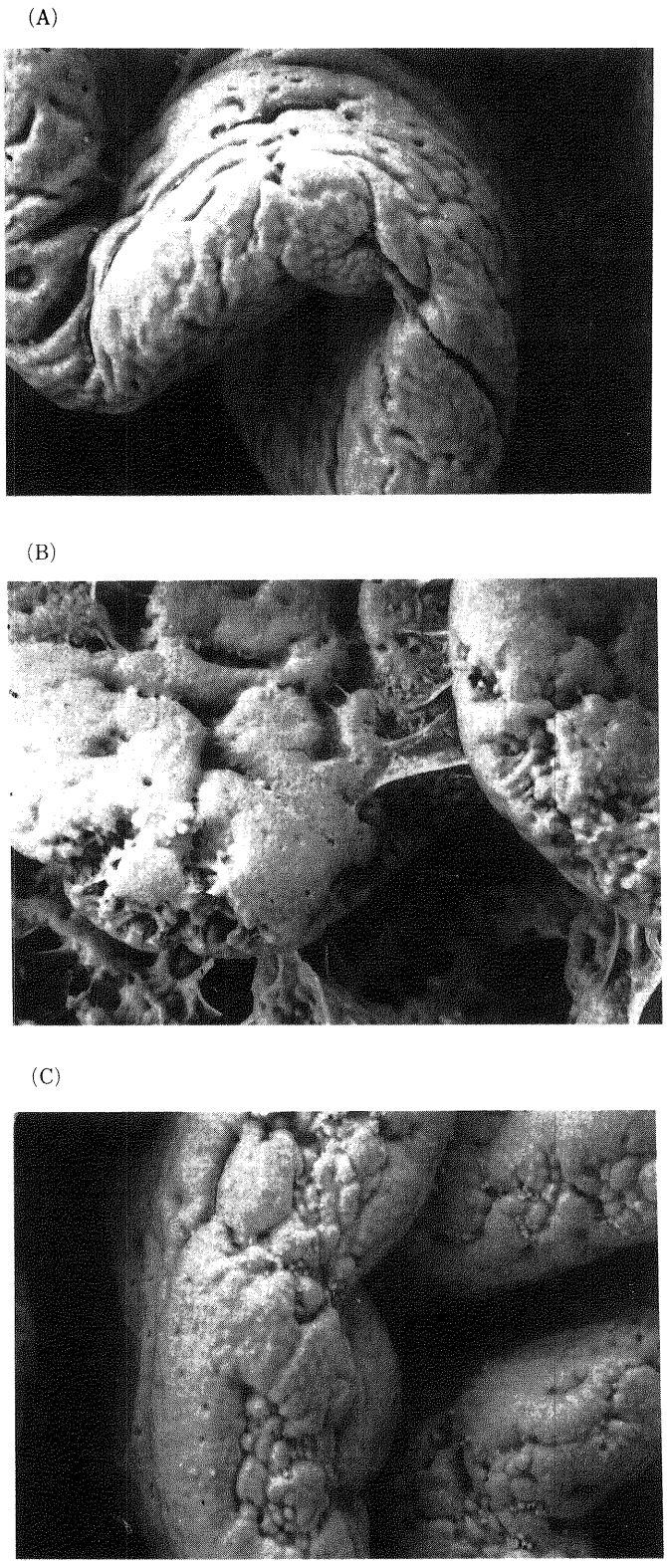


Fig. 2. Scanning Electron Micrographs of Intestinal Mucosa in Rabbits 48 h after Oral Administration of 5-FU (30 mg/kg)

(A) duodenum, (B) jejunum, (C) ileum. Magnification: ×230.

morphological change of the ileal mucosa compared with the control was observed following the oral pretreatment with 5-FU.

In the present study, SAM was used as a marker compound for an assessment of the mucosal damage in the intestine. SAM, a mild analgesic and antipyretic agent, was reported to exhibit first-pass metabolism during intestinal

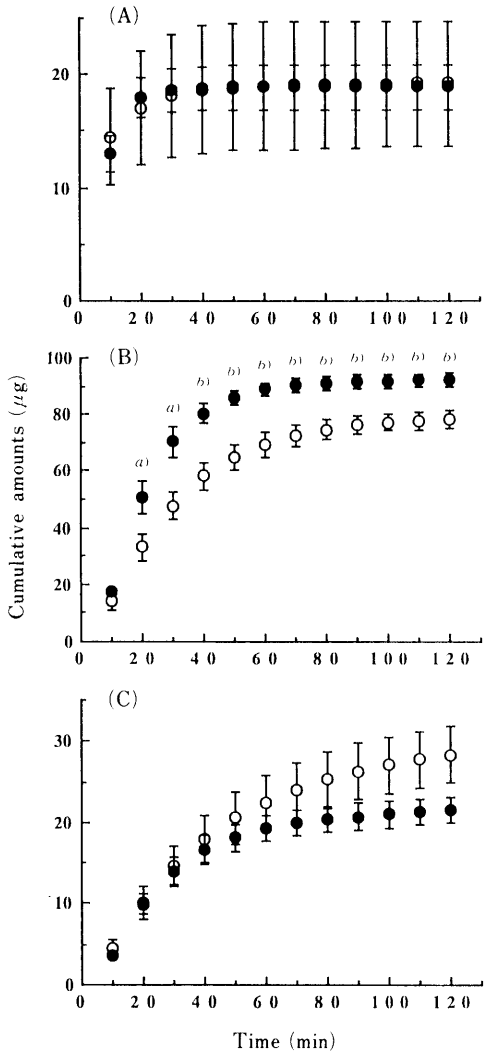


Fig. 3. Appearance of SAM and Its Metabolites in Mesenteric Venous Blood after an Injection of SAM in the Intestinal Lumen

(A) SAM, (B) SAMG, (C) SAMS. Key: (○) control (5), (●) 5-FU pretreatment orally (5). Dose: 3 ml of 67 μg/ml solution of SAM. The amounts of SAMG and SAMS were calculated as SAM. Results are expressed as the mean ± S.E. Numbers in parentheses represent number of experiments. Statistical significance: *a*) $p<0.05$, *b*) $p<0.01$. The results of the control were reported previously.[27]

absorption in rabbits,[10,25-27] rats[12,28-32] and dogs.[33-36]

The effect of oral pretreatment with 5-FU 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 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.

Figure 3 shows the appearance of SAM (A), SAMG (B) and SAMS (C) in the mesenteric venous blood after an injection of SAM into the intestinal lumen. Following the oral pretreatment with 5-FU, no effect was found in the appearance of SAM compared with the control (Fig. 3A). 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 3B shows the appearance of SAMG in the mesenteric venous blood. Following oral pretreatment with 5-FU, the appearance of

Table I. SAM and Its Metabolites in the Intestinal Luminal Solution in 120 min

| Pretreatment | SAM | SAMG | SAMS | Total |
|---|---|---|---|---|
| | | ($\mu$g) | | |
| Control (5) | 1.1±0.6 | 23.3±1.8 | 16.9±1.8 | 41.3±3.9 |
| 5-FU (5) | 0.3±0.2 | 20.5±5.9 | 13.4±2.1 | 34.2±6.1 |

Dose: 3 ml of 67 $\mu$g/ml solution of SAM. Each value is expressed as the mean±S.E. Numbers in parentheses represent number of experiments. The amounts of SAMG and SAMS were calculated as SAM. The results of the control were reported previously.[27]

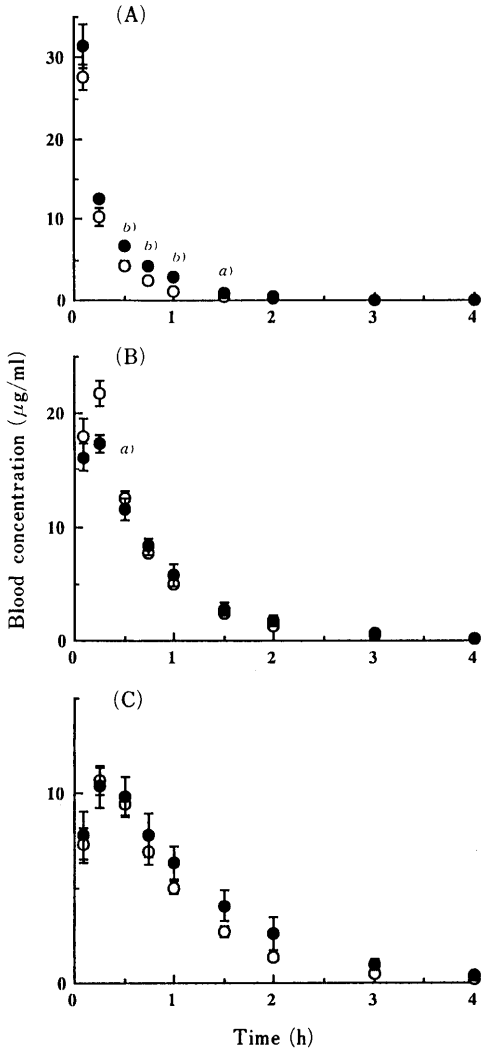


Fig. 4. Blood Concentration of SAM and Its Metabolites Following Intravenous Administration of SAM in Rabbits

(A) SAM, (B) SAMG, (C) SAMS. Key: (○) control (6), (●) 5-FU pretreatment orally (5). Dose: 30 mg/kg of SAM. The blood concentration of SAMG and SAMS were calculated as SAM. Results are expressed as the mean±S.E. Numbers in parentheses represent number of experiments. Statistical significance: *a*) $p<0.05$, *b*) $p<0.01$. The results of the control were reported previously.[37]

SAMG increased from 20 to 120 min compared with the control. As shown in Fig. 3C, 5-FU pretreatment tended to decrease the appearance of SAMS in 120 min compared with the control. However, no effect was observed in the appearance of SAMS in the mesenteric venous blood at the beginning of the absorption period. These results indicate the alteration of SAM metabolism in the intestinal mucosa.

To provide more information on the intestinal first-pass metabolism of SAM, SAM and its metabolites in the

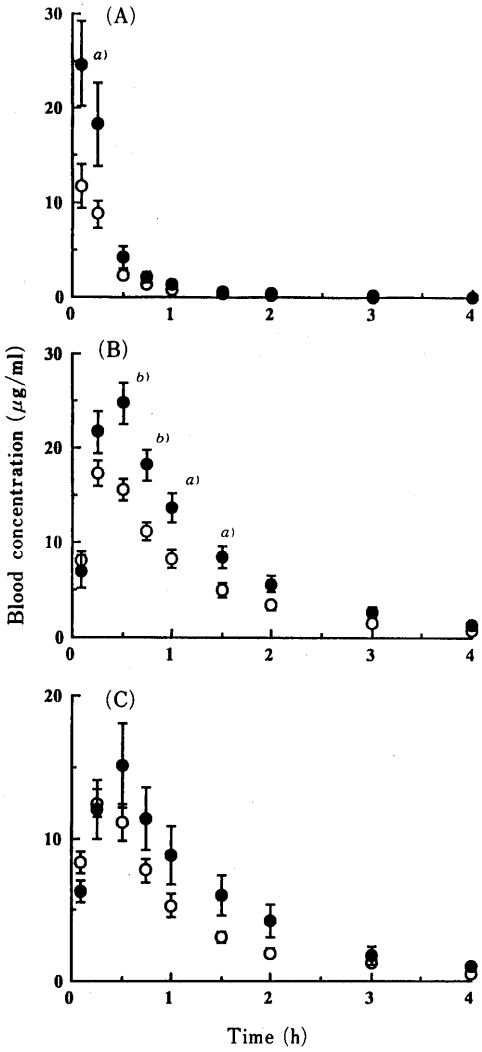


Fig. 5. Blood Concentration of SAM and Its Metabolites Following Intraduodenal Administration of SAM in Rabbits

(A) SAM, (B) SAMG, (C) SAMS. Key: (○) control (5), (●) 5-FU pretreatment orally (6). Dose: 30 mg/kg of SAM. The blood concentration of SAMG and SAMS were calculated as SAM. Results are expressed as the mean±S.E. Numbers in parentheses represent number of experiments. Statistical significance: *a*) $p<0.05$, *b*) $p<0.01$. The results of the control were reported previously.[37]

intestinal luminal solution at 120 min after an injection of SAM were determined. The results are presented in Table I. In control rabbits, 1.1 $\mu$g of SAM (0.5% of dose) appeared in the intestinal luminal solution. Both the control and 5-FU-pretreated rabbits showed almost complete absorption of SAM. A significant effect of 5-FU pretreatment on the appearance of SAMG and SAMS in the intestinal luminal solution was not observed.

In order to examine the effect of oral pretreatment with 5-FU on the distribution and elimination patterns of SAM, the blood concentration of SAM and its metabolites after intravenous administration of SAM was determined. The results are presented in Fig. 4. As shown in Fig. 4A, the blood concentration of SAM in rabbits pretreated with 5-FU orally increased slightly from 30 to 90 min. In Fig. 4B, a significant effect was not found in the blood concentration of SAMG, except for a decreased blood concentration of SAMG in 30 min. Both control and 5-FU-pretreated rabbits showed an almost identical blood concentration of SAMS in Fig. 4C.

To assess the mucosal damage induced by oral

pretreatment with 5-FU *in vivo*, the blood concentration of SAM and its metabolites was determined following the intraduodenal administration of SAM. The results are shown in Fig. 5. As shown in Fig. 5A, the blood concentration of SAM in rabbits pretreated with 5-FU orally was significantly increased in 5 min, suggesting the increased permeability of SAM. In Fig. 5B, a significant increase was recognized in the blood concentration of SAMG from 30 to 90 min. In rabbits pretreated with 5-FU orally, the blood concentration of SAMS tended to increase compared with the control.

In the previous reports, we demonstrated a change in the intestinal first-pass metabolism of SAM in rabbits pretreated with indomethacin[27] or salicylic acid[37] orally, suggesting the change was due to intestinal mucosal damage. From the results described above, it is suggested that the change in intestinal first-pass metabolism of SAM may be due to the mucosal damage in the intestine by oral pretreatment with 5-FU. Nothing definite is known about the mechanism by which the mucosal damage causes the change of SAM metabolism in the intestine. Further studies are needed to investigate the urinary recovery of a marker compound and its metabolites following oral administration. The alteration of the intestinal first-pass metabolism of a marker compound may be utilized as a convenient and non-invasive screening test for quantitative assessment of intestinal mucosal damage *in vivo*. In addition, this test may be helpful both in diagnosis and in assessment of responses to the treatment of intestinal mucosal damage.

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