An Assessment of Indomethacin-Induced Mucosal Damage *in Vivo* by Measuring the Metabolism of Salicylamide in Rabbit Intestine

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Indomethacin-induced mucosal damage was assessed *in vivo* by measuring salicylamide (SAM) metabolism in rabbit intestine. Intestinal mucosal damage 48 h after oral indomethacin (500 mg/kg) administration was examined using a scanning electron microscope. Duodenal, jejunal and ileal mucosal toxicity was compared with that in controls. Intestinal first-pass metabolism of SAM was studied using *in situ* intestinal sacs with intact mesenteric venous blood collection. The appearance of both SAM and its metabolites in the mesenteric venous blood was measured following cannulation of the mesenteric vein of the exposed intestine and collecting all venous blood draining from the absorbing region. Following oral pretreatment with indomethacin, the appearance of SAM and SAM glucuronide (SAMG) in the mesenteric venous blood was significantly increased. The concentrations of SAM and SAMG in the blood increased following intraduodenal administration of SAM *in vivo* in rabbits orally pretreated with indomethacin compared with controls. However, after intravenous administration of SAM, the blood concentration of SAM and SAMG was not increased compared with controls. These findings suggest that the differences in intestinal first-pass metabolism of SAM may be due to the intestinal mucosal damage induced by oral indomethacin pretreatment. The results indicate that the alteration of intestinal first-pass metabolism of a marker compound may be utilized to assess intestinal mucosal damage *in vivo*.

Keywords mucosal damage; intestinal metabolism; indomethacin; salicylamide; rabbit; intestinal absorption; screening test; membrane permeability; membrane transport; assessment

The gastrointestinal tract is an important site for side effects of drugs. Indomethacin has prominent antiinflammatory and analgesic-antipyretic properties. Although indomethacin is widely used and is effective, toxicity often limits its use. Gastrointestinal complaints and complications consist of anorexia, nausea and abdominal pain. Single ulcers or multiple ulceration of the entire upper gastrointestinal tract, sometimes with perforations and hemorrhage, have been reported.[1] Diarrhea may occur and is sometimes associated with ulcerative bowel lesions. Gastrointestinal mucosal damage is usually assessed by macroscopical and microscopical examination. Measurement of gastrointestinal blood loss is also used extensively. Several studies have investigated gastrointestinal mucosal damage by assessing the permeability of low molecular weight polyethylene glycols,[2-4] cellobiose and mannitol,[5-7] ^{51}chromium-labeled ethylenediaminetetraacetate[8] and phenolsulfonphthalein.[9-12] In previous reports from this laboratory, we examined mucosal damage induced by oral pretreatment with 5-fluorouracil[13] or salicylic acid[14] by measuring the metabolism of salicylamide (SAM) as a marker compound in rabbit intestine *in vivo*. The present study was undertaken to investigate mucosal damage induced by oral pretreatment with indomethacin by measuring the metabolism of SAM in rabbit intestine *in vivo*.

Experimental

Materials SAM and glutaraldehyde (25% in water) were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Indomethacin was obtained from Sigma Chemical Co. (St. Louis, MO., U.S.A.), carboxymethylcellulose sodium salt (CMC) from Hayashi Pure Chemicals 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 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 were 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. Forty eight hours later, scanning electron micrography of the intestinal mucosa, *in situ* absorption experiments and *in vivo* absorption experiments were carried out after fasting the rabbits for 24 h. During fasting, animals were allowed free access to water.

Scanning Electron Micrography of Intestinal Mucosa The intestinal tracts of at least 2 rabbits were 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 1% glutaraldehyde solution diluted with pH 7.3 phosphate buffer solution to fix over 1 h at 4 °C. The 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[15] with slight modifications.[16] Results were compared statistically using 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 using a heparinized syringe at appropriate time intervals from the vein of another ear.

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 with a heparinized syringe at appropriate time intervals from an ear vein.

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.*[17] 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

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

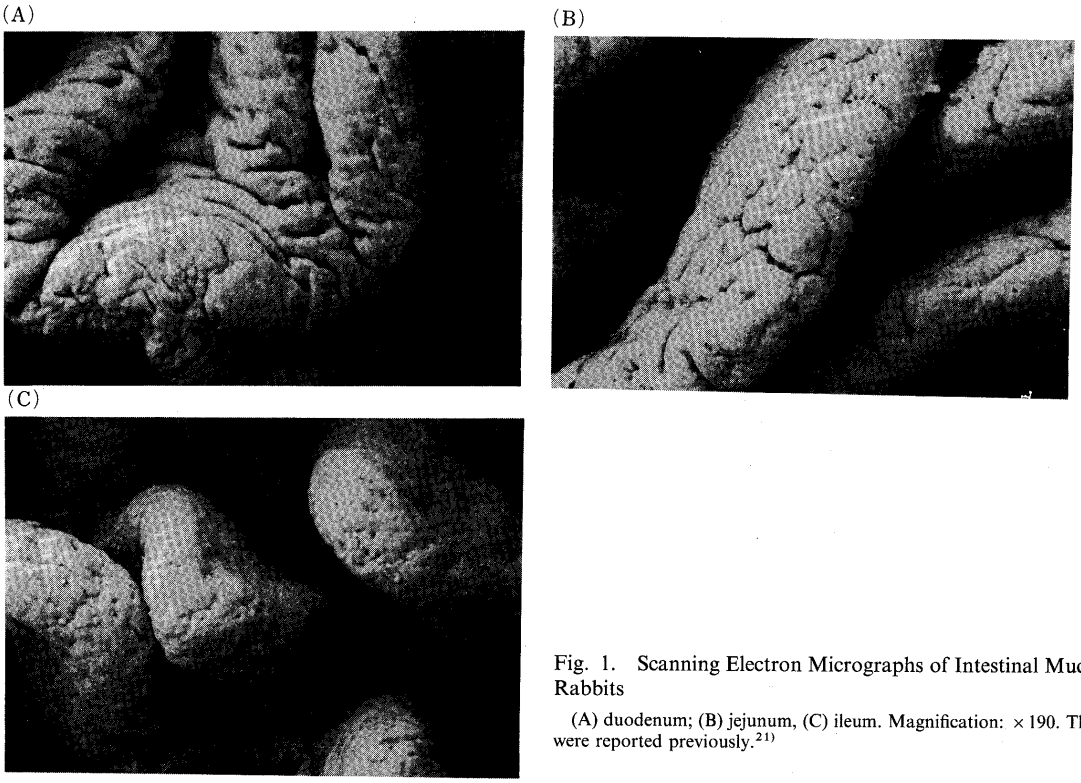

Fig. 1. Scanning Electron Micrographs of Intestinal Mucosa in Control Rabbits

(A) duodenum; (B) jejunum, (C) ileum. Magnification: × 190. These micrographs were reported previously.[21]

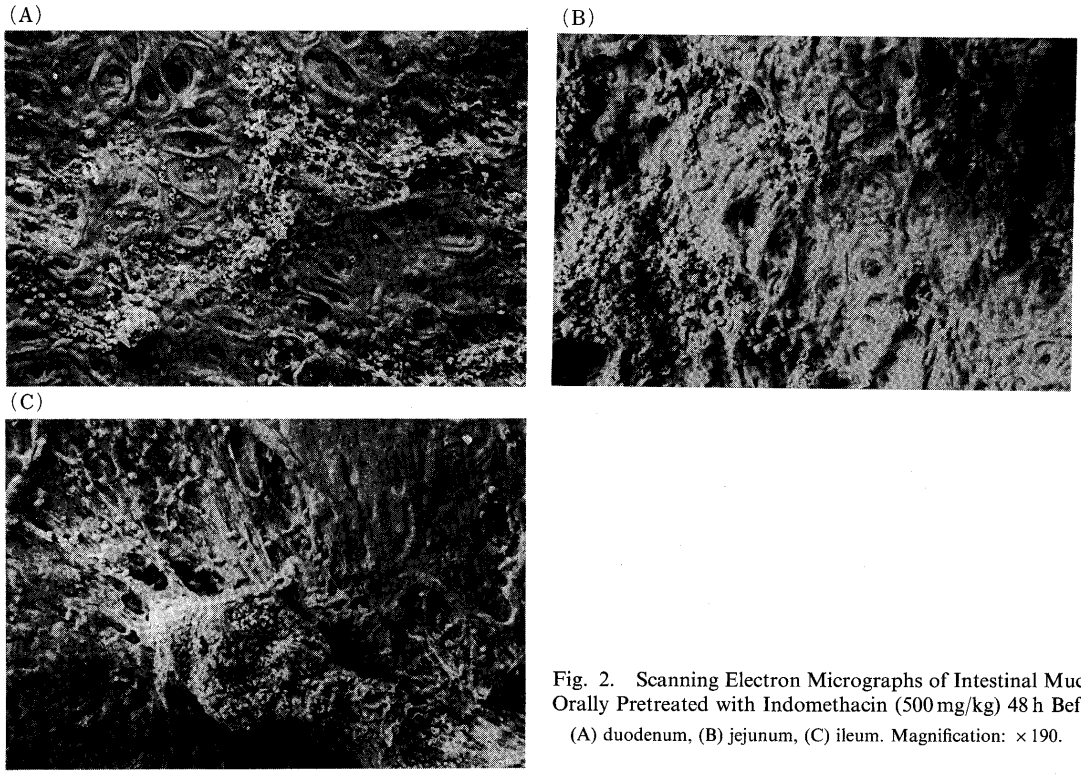

Fig. 2. Scanning Electron Micrographs of Intestinal Mucosa in Rabbits Orally Pretreated with Indomethacin (500 mg/kg) 48 h Before

(A) duodenum, (B) jejunum, (C) ileum. Magnification: × 190.

discriminated at this magnification. Tongue-shaped villi were found in the jejunum (Fig. 1B). In Fig. 1C, can be seen ileal villi which were broad and tongue-shaped structures.

Figure 2 shows scanning electron micrographs of the intestinal mucosa in rabbits pretreated with indomethacin orally 48 h earlier. Severe damage of duodenal (Fig. 2A), jejunal (Fig. 2B) and ileal (Fig. 2C) mucosa was observed. Somogyi *et al.*,[18] Kent *et al.*[19] and Brodie *et al.*[20] reported that indomethacin administration caused extensive lesions characterized by peritonitis, ulceration and occasional frank necrosis in the lower small intestine in rats.

The effect of oral indomethacin pretreatment on the intestinal first-pass metabolism of SAM was examined in rabbits using *in situ* intestinal sacs with intact mesenteric venous blood collection. SAM is metabolized to SAMG

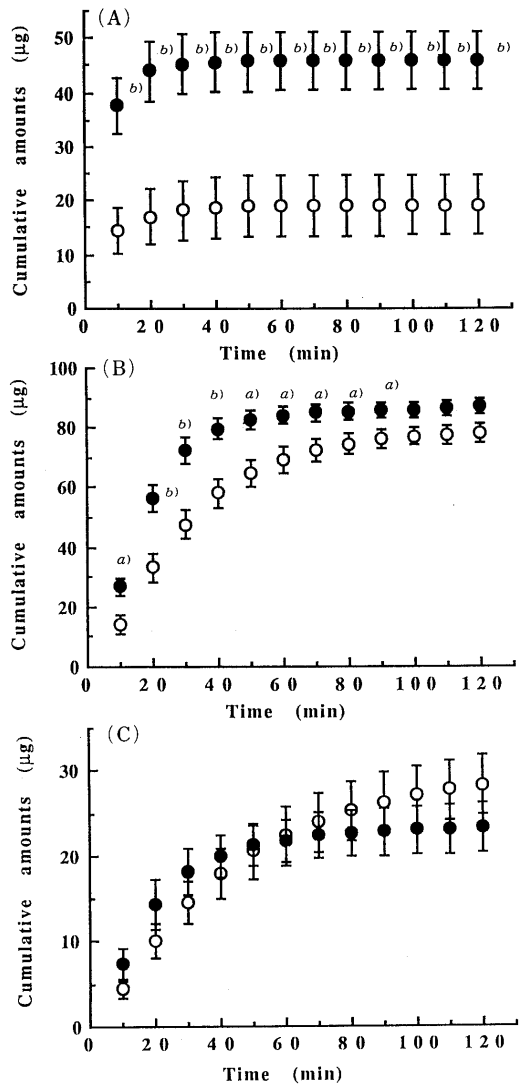

Fig. 3. Appearance of SAM and Its Metabolites in Mesenteric Venous Blood after an Injection of SAM in the Intestinal Lumen

(A) SAM, (B) SAMG, (C) SAMS. Key: (○) control (5), (●) oral pretreatment with indomethacin (500 mg/kg) 48 h before (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 control results were reported previously.[22]

and SAMS in the rabbit intestine. Minor metabolites of SAM were not determined in this study.

Figure 3 shows the appearance of SAM (Fig. 3A), SAMG (Fig. 3B) and SAMS (Fig. 3C) in the mesenteric venous blood after an injection of SAM into the intestinal lumen. As shown in Fig. 3A, oral indomethacin pretreatment resulted in enhanced SAM appearance compared with controls. The cumulative amounts of SAM in the mesenteric venous blood tended to plateau in 30 min, suggesting the rapid intestinal absorption of SAM. Figure 3B shows the appearance of SAMG in the mesenteric venous blood. The appearance of SAMG increased at the beginning of the absorption period compared with controls. However, no effect was found in cumulative amounts of SAMG from 100 to 120 min. As shown in Fig. 3C, no effect was observed in the appearance of SAMS compared with controls.

To provide more information on the intestinal first-pass metabolism of SAM, SAM and its metabolites in the intestinal luminal solution were determined after an injection of SAM. The results are shown in Table I. In control rabbits, 1.1 μg of SAM (0.5% of dose) remained in the intestinal luminal solution. On the other hand, 0.3 μg of SAM (0.1% of dose) remained in the intestinal luminal solution in rabbits orally pretreated with indomethacin.

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

Pretreatment (*n*)	SAM	SAMG	SAMS	Total
		(μg)		
Control (5)	1.1±0.6	23.3±1.8	16.9±1.8	41.3±3.9
Indomethacin (5)	0.3±0.3	21.8±2.7	5.9±1.1[b]	28.0±3.3[a]

Indomethacin (500 mg/kg) was administered orally 48 h before. Dose: 3 ml of 67 μg/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. Statistical significance: *a*) $p<0.05$, *b*) $p<0.001$. The control results were reported previously.[22]

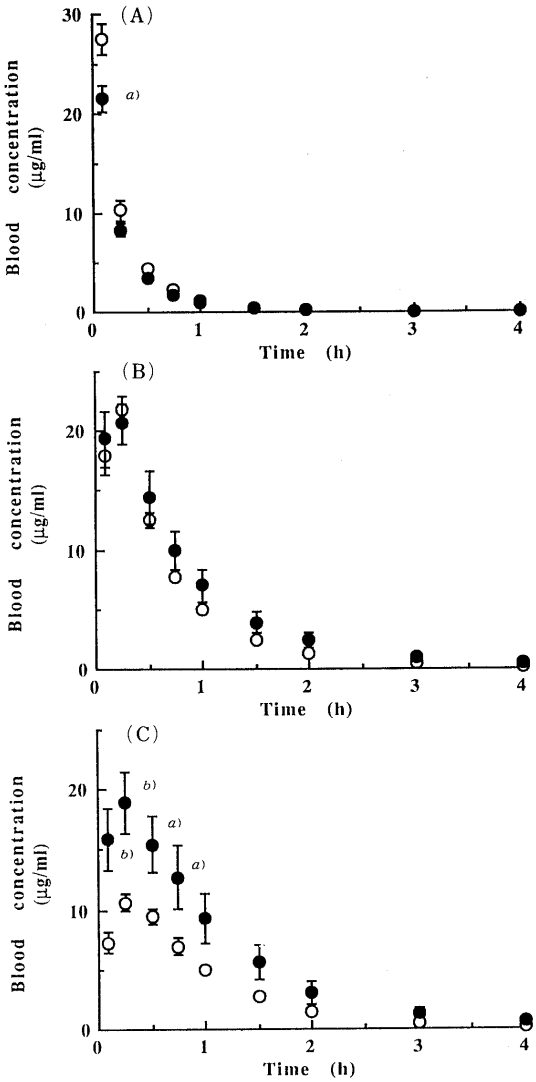

Fig. 4. Blood Concentration of SAM and Its Metabolites Following Intravenous Administration of SAM in Rabbits

(A) SAM, (B) SAMG, (C) SAMS. Key: (○) control (6), (●) oral pretreatment with indomethacin (500 mg/kg) 48 h before (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 control results were reported previously.[14]

These results show almost complete disappearance of SAM from the luminal solution both in control and in indomethacin-pretreated rabbits. A significant decrease of SAMS appearance in the intestinal luminal solution was observed in rabbits pretreated with indomethacin orally. Additional studies are needed to investigate the mechanisms responsible for this decrease. However, no effect was found on the appearance of SAMG in the intestinal luminal solution.

In order to examine the effect of oral pretreatment with indomethacin on the distribution and elimination patterns of SAM, the blood concentrations of SAM and its metabolites were determined following intravenous administration of SAM. The results are shown in Fig. 4. In Fig. 4A, the blood concentration of SAM in rabbits orally pretreated with indomethacin did not change compared with controls, except for decreased SAM blood concentrations at 5 min. Control and oral indomethacin-pretreated rabbits exhibited almost identical blood concentration of SAMG in Fig. 4B. As shown in Fig. 4C, however, the blood concentration of SAMS in rabbits pretreated with indomethacin orally was significantly increased from 5 to 45 min.

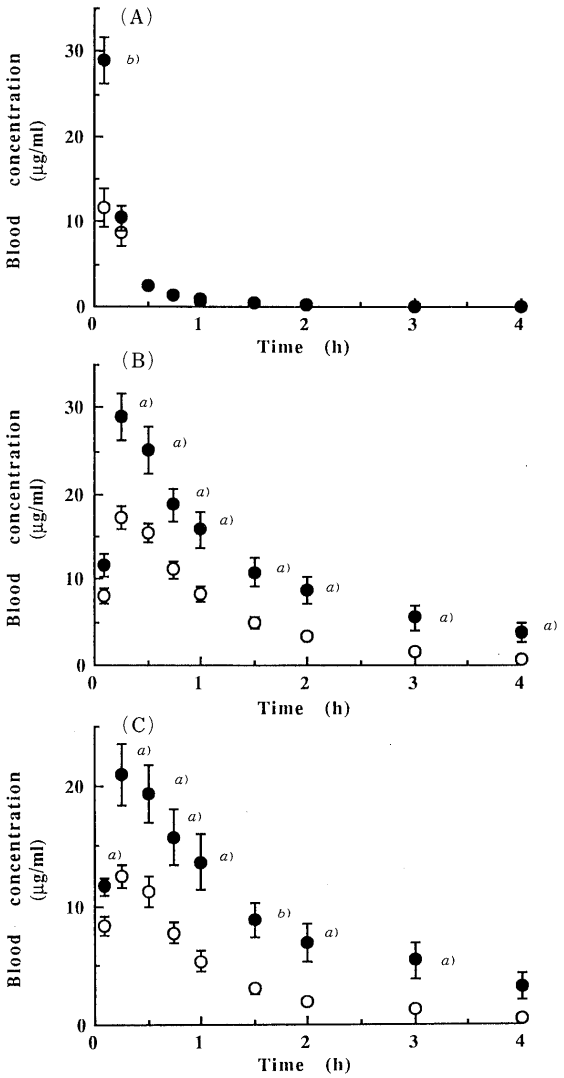

Fig. 5. Blood Concentration of SAM and Its Metabolites Following Intraduodenal Administration of SAM in Rabbits

(A) SAM, (B) SAMG, (C) SAMS. Key: (○) control (5), (●) oral pretreatment with indomethacin (500 mg/kg) 48 h before (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 control results were reported previously.[14]

To assess the intestinal mucosal damage induced by oral pretreatment with indomethacin *in vivo*, the blood concentration of SAM and its metabolites was determined after the intraduodenal administration of SAM. The results are presented in Fig. 5. In Fig. 5A, a significant increase of the SAM blood concentration in rabbits orally pretreated with indomethacin was observed at 5 min. As shown in Fig. 5B, the SAMG blood concentrations in rabbits orally pretreated with indomethacin were significantly increased from 15 min to 4 h, suggesting an alteration of intestinal SAM metabolism. In rabbits orally pretreated with indomethacin, an increased SAMS blood concentration was observed. Results of *in situ* absorption experiments and intravenous administration experiments suggest that the increased SAM blood concentration observed following intraduodenal SAM administration may be due to an altered hepatic SAM metabolism.

In prior reports, we also demonstrated altered intestinal first-pass metabolism of SAM in rabbits orally pretreated with 5-fluorouracil[13] or salicylic acid[14] using *in situ* intestinal sacs with intact mesenteric venous blood collection. Furthermore, an increased SAMG blood concentration following intraduodenal administration of SAM *in vivo* was shown in rabbits orally pretreated with 5-fluorouracil or salicylic acid. However, the blood concentration of SAMG after intravenous administration of SAM was not increased compared with controls.

Based on these considerations, it is suggested that a change in intestinal first-pass metabolism of SAM may be produced due to mucosal damage in the intestine following oral pretreatment with 5-fluorouracil, salicylic acid or indomethacin. In the present study, we did not examine the effect of indomethacin on drug-metabolizing enzymes in the intestine. Therefore, nothing definite can be reported at this time concerning the mechanism by which the mucosal damage causes a change of SAM metabolism in the intestine. Further studies are needed to investigate the blood concentration and the urinary recovery of SAM and its metabolites following oral administration for diagnostic use. Thus the alteration of intestinal first-pass metabolism of a marker compound such as SAM may be utilized as a convenient and noninvasive 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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