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Protection by aspirin of indomethacin-induced small intestinal damage in rats: mediation by salicylic acid

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Abstract

Most of non-steroidal anti-inflammatory drugs (NSAIDs) except aspirin (ASA) produce intestinal damage in rats. In the present study, we re-examined the intestinal toxic effect of ASA in rats, in comparison with various NSAIDs, and investigated why ASA does not cause damage in the small intestine, in relation to its metabolite salicylic acid (SA). Various NSAIDs (indomethacin; 10 mg/kg; flurbiprofen; 20 mg/kg; naproxen; 40 mg/kg; dicrofenac; 40 mg/kg; ASA; 20–200 mg/kg) were administered s.c., and the small intestinal mucosa was examined macroscopically 24 h later. All NSAIDs tested, except ASA, caused hemorrhagic lesions in the small intestine, with a decrease of mucosal PGE_2 contents. ASA did not provoke any damage, despite inhibiting (prostaglandin) PG production, and prevented the occurrence of intestinal lesions induced by indomethacin, in a dose-related manner. This protective action of ASA was mimicked by the equimolar doses of SA (17.8–178 mg/kg). Indomethacin caused intestinal hypermotility, in preceding to the ocurrence of lesion, and this event was followed by increases of enterobacterial translocation in the mucosa. Both ASA and SA prevented both the intestinal hypermotility and the bacterial translocation seen after indomethacin treatment. In addition, the protective effect of SA was not significantly influenced by either the adenosine deaminase or the adenosine receptor antagonists. Following administration of ASA, the blood SA levels reached a peak within 30 min and remained elevated for more than 7 h. These results suggest that SA has a cytoprotective action against indomethacin-induced small intestinal lesions, and this action may be associated with inhibition of the intestinal hypermotility and the bacterial translocation, but not mediated by endogenous adenosine. Failure of ASA to induce intestinal damage may be explained, at least partly, by a protective action of SA, the metabolite of ASA.

Keywords: Aspirin; Intestinal lesion; Salicylic acid; Non-steroidal anti-inflammatory drug; Prostaglandin; Intestinal motility; Rat

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) such as indomethacin causes intestinal damage as a side effect in human or experimental animals [3,8,15]. Although the inhibition of cyclo-oxygenase, leading to depletion of endogenous prostaglandins (PGs), plays as a major papthogenic element in the development of these lesions [22], other factors including bacterial flora, nitric oxide (NO), neutrophil and oxyradicals may also be involved in their pathogenesis [4,8,10,14,19,21,23,24].

On the other hand, it is known that aspirin (ASA), unlike other NSAIDs, does not provoke damage in the small intestine [15,18]. The failure of this agent to induce intestinal damage has been explained by the lack of enterohepatic circulation of this agent [4], yet the exact mechanism remains unexplored. Of interest, ASA does not also produced damage in the stomach when administered parenterally, despite decreasing the mucosal PG production [19]. Robert et al. [15,16] reported that ASA showed an anti-ulcer effect against various experimental ulcer models including gastrointestinal lesions. We recently confirmed the protective action of ASA against indomethacin-induced gastric lesions and further found that this effect may be brought about by a cytoprotective action of salicylic acid (SA), the metabolite of ASA [19]. On mere speculation, it is possible that the same might be applied to the action of ASA on the small intestinal mucosa, that is, if SA is cytoprotective in the intestine, this might explain why ASA does not show the ulcerogenic property in the small intestine.

In the present study, we re-examined the ulcerogenic effect of ASA on the small intestinal mucosa, in comparison with other conventional NSAIDs, and investigated whether ASA or SA protects the small intestine against indomethacin-induced damage. In addition, since recent studies suggest that the anti-inflammatory action of SA is mediated, at least partly, by endogenous

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adenosine [5–7], we also examined the possible involvement of adenosine in the protective action of SA in the small intestine.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley (SD) rats (200–220 g) were used without fasting. All studies were performed using 4–6 animals per group under unanesthetized conditions, unless otherwise specified. The experimental procedures employed in the present study were approved by the Experimental Animal Research Committee of the Kyoto Pharmaceutical University.

2.2. Induction of small intestinal lesions

The animals were administered various NSAIDs, such as indomethacin (10 mg/kg), flurbiprofen (20 mg/kg), naproxen (40 mg/kg), dicrofenac (40 mg/kg), ASA (200 mg/kg) s.c., killed 24 h later under deep ether anesthesia, and both the jejunum and ileum were removed and treated with 2% formalin for fixation of the tissues. Then, they were opened along the mesenteric attachment and examined for lesions under a dissecting microscope with square grids (×10). The area (mm^2) of hemorrhagic lesions was measured, summed per small intestine, and used as a lesion score. The person measuring the lesions did not know the treatments given to the animals. Some of the animals given indomethacin were pretreated s.c. with ASA (20–200 mg/kg) or SA (17.8–178 mg/kg) 30 min before indomethacin. In some cases, the animals given SA (48 mg/kg, s.c.) were also pretreated with the following drugs, in order to investigate the involvement of adenosine in the protective action of SA; 8-phenyltheophylline (8-PT: the adenosine A1 receptor antagonist, 10 mg/kg, s.c.), 3,7,-dimethyl-1-propargylxanthine (DMPX: the adenosine A2 receptor antagonist, 10 mg/kg, s.c.) or adenosine deaminase (75 U/kg, i.v.) was given 30 or 10 min before the administration of SA, respectively. The doses of these drugs were chosen to mitigate the anti-inflammatory action of SA through deamination of adenosine or antagonizing the adenosine receptors [7].

2.3. Measurement of mucosal prostaglandin E_2 levels

PGE_2 levels in the small intestinal mucosa were measured at 6 h after administration of various NSAIDs (indomethacin; 10 mg/kg; flurbiprofen; 20 mg/kg; naproxen; 40 mg/kg; dicrofenac; 40 mg/kg; ASA; 200 mg/kg). Under ether anesthesia, the small intestine was removed, and the mucosa was isolated, weighed, and put in a tube containing 100% ethanol plus 0.1 M indomethacin [9]. Then, the samples were minced by scissors, homogenized, and centrifuged for 10 min at 12,000 rpm at 4 °C. The supernatant of each sample was used for determination of PGE_2 by EIA using PGE_2-kit (Cayman Chemical Co., Ann Arbor, MI, USA).

2.4. Measurement of bacterial translocation

Twenty-four hours after administration of indomethacin (10 mg/kg), the animals were killed under deep ether anesthesia, and the small intestines were removed. After rinsing the intestine with sterile saline, the mucosa was scraped with glass slides, weighed, and homogenized in 1 ml of sterile phosphate buffer saline (PBS) per 100 mg wet tissue. The aliquot of the homogenate was placed on blood agar and GAM agar (Nissui, Osaka, Japan). Blood agar plates were incubated at 37 °C for 24 h under aerobic conditions, while GAM agar plates were incubated for 48 h under standard anaerobic conditions (BBL Gas Pack Pouch Anaerobic System, Becton Deckinson, Maryland, USA). Plates containing 10–200 colony-forming units (CFU) were examined for enterobacterial numbers invaded in the small intestine, and the data were expressed as log CFU/g tissue. ASA (60 mg/kg) or SA (53.4 mg/kg) were given s.c. 30 min before indomethacin treatment.

2.5. Determination of intestinal motility

Intestinal motility was determined using a miniature balloon according to a previous paper [11]. In brief, the rat was anesthetized with urethane (1.25 g/kg, i.p.), and the trachea was cannulated to facilitate respiration. Following a midline incision to expose the small intestine, a thin, saline-filled balloon, made from silicone rubber and attached to a polyethylene catheter, was introduced into the jejunum via a small incision and tied in place avoiding large blood vessels. The volume in the balloon was adjusted to give an initial resting pressure of 5 mmHg, which was not sufficient to cause active distension of the intestinal wall, and after allowing the preparation to rest for 30 min, intestinal motility was monitored on a recorder (U-228, Tokai-irika, Tokyo, Japan) as intraluminal pressure changes, through a pressure transducer and polygraph device (Nikon Kodan, Ibaragi, Japan). Indomethacin (10 mg/kg) was given s.c. after basal motor activity had well stabilized, while ASA (60 mg/kg) or SA (53.4 mg/kg) was given s.c. 2 h after administration of indomethacin.

2.6. Measurement of blood levels of salicylic acid

Plasma SA levels were determined following s.c. administration of ASA (200 mg/kg), according to the modified method of Urushidani et al. [20] Three hundred microliters of blood was collected from the tail

vein in the presence of heparin, at 15, 30, 45, 60, 180 and 420 min after administration of these drugs. Blood samples were centrifuged at 10,000 rpm for 5 min at 4 °C and stored at −80 °C until the assay. For determination of SA levels, the samples (100 μl) were mixed with 20 μl of 6 M HCl, boiled at 100 °C for 10 min, and shaken with 600 μl of ethylene dichloride for 5 min. After extraction, the organic phase was transferred to another tube. Then, 200 μl of 1.76% of ferric nitrate was added, and the mixture was shaken for 5 min. The aqueous layer was collected, and the SA levels were measured by spectrophotometry at 545 nm. A standard curve was made using the rat plasma containing various concentrations of SA.

2.7. Preparation of drugs

Drugs used were indomethacin, ASA, SA, flurbiprofen, naproxen, dicrofenac, 8-PT, adenosine deaminase (Sigma Chemicals, St. Louis, MO, USA), 3,7,-dimethyl-1-propargylxanthine (DMPX: Research Biochemicals Inter, Natric, MA, USA) and urethane (Nacalai tesque, Kyoto, Japan). All NSAIDs were suspended in saline with a drop of Tween 80 (Wako), while other drugs were dissolved in saline. All drugs were prepared immediately before use and administered s.c. or i.p. in a volume of 0.5 ml/100 g body weight.

2.8. Statistics

Data were presented as the means±S.E. from 4 to 6 rats per group. Statistical analyses were performed using two-tailed Dunnett's multiple comparison test, and values of $P<0.05$ were considered as significant.

3. Results

3.1. Effects of various NSAIDs on small intestinal mucosa and PGE_2 contents

Subcutaneous administration of NSAIDs tested, except ASA, provoked lesions in the small intestinal mucosa within 24 h; the lesion score being 211.8±21.7, 189.8±30.3, 212.6±29.9, and 145.4±21.0 mm^2, respectively, for indomethacin (10 mg/kg), naproxen (40 mg/kg), flurbiprofen (20 mg/kg) or diclofenac (20 mg/kg; Fig. 1). ASA at 200 mg/kg did not cause any damage in the intestine. However, all NSAIDs at the doses used, even including ASA, caused a marked decrease in the mucosal PGE_2 contents in the small intestine (Fig. 2). The mucosal PGE_2 contents (27.0±3.9 pg/mg tissue) in control animals the levels decreased below 3 pg/mg tissue when determined 6 h after administration of these agents, and the inhibitory effect ASA on PGE_2 production was equivalent to those induced by other NSAIDs.

3.2. Effects of ASA and SA on indomethacin-induced small intestinal damage and bacterial translocation

3.2.1. Lesions

Subcutaneous administration of indomethacin (10 mg/kg) caused hemorrhagic lesions in the small intestine within 24 h; the lesion score was 207.8±26.5 mm^2. When the animals were pretreated with ASA (20–200 mg/kg) s.c. 30 min before indomethacin, the development of these lesions was prevented in a dose-dependent manner, and a significant effect was observed at over 60

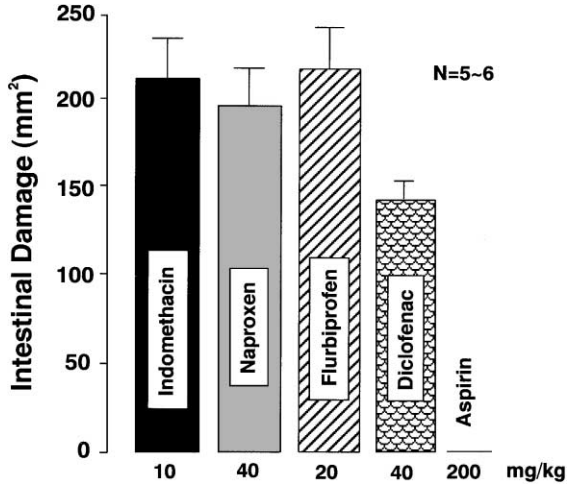

Fig. 1. Small intestinal ulcerogenic responses induced by various non-steroidal anti-inflammatory drugs (NSAIDs) in rats. The animals were given indomethacin (10 mg/kg), naproxen (40 mg/kg), flurbiprofen (20 mg/kg), dicrofenac (40 mg/kg) and aspirin (ASA; 200 mg/kg) s.c., and they were killed 24 h later. Data were presented as the means±S.E. from 5 to 6 rats.

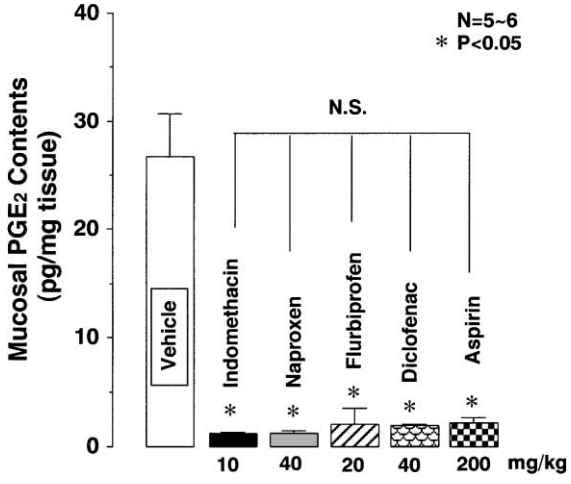

Fig. 2. Effects of various non-steroidal anti-inflammatory drugs (NSAIDs) on the mucosal PGE_2 contents in the rat small intestine. The animals were given indomethacin (10 mg/kg), naproxen (40 mg/kg), flurbiprofen (20 mg/kg), dicrofenac (40 mg/kg) and aspirin (ASA; 200 mg/kg) s.c., and they were killed 6 h later. Data were presented as the means±S.E. from 5 to 6 rats. *Significant difference from control, at $P<0.05$.

mg/kg (Fig. 3), the inhibition at 200 mg/kg being 94.1%. Likewise, the pretreatment of the animals with SA (17.8–178 mg/kg), the equimolar doses of ASA, also reduced the severity of indomethacin-induced intestinal lesions in a dose-dependent manner, the inhibition at 53.4 and 178 mg/kg being 85.6 and 100%, respectively. Certainly, either of these agents alone at any doses did not provoke damage in the small intestine (not shown).

3.2.2. Bacterial translocation

The aerobic and anaerobic enterobacterial numbers in the normal intestinal mucosa were 7.31±0.28 log CFU/g tissue and 7.64±0.25 log CFU/g tissue, respectively (Fig. 4). Following s.c. administration of indomethacin (10 mg/kg), the enterobacterial numbers in both aerobic and anaerobic were markedly increased to approximately 1000-fold greater than controls, the values being 9.97±0.16 and 10.15±0.15 log CFU/g tissue, respectively. Prior administration of ASA (60 mg/kg) or SA (53.4 mg/kg) suppressed the increase of bacterial translocation in the mucosa in response to indomethacin, and the values in both aerobic and anaerobic bacterium were significantly decreased as compared with those seen in the animals treated with indomethacin alone.

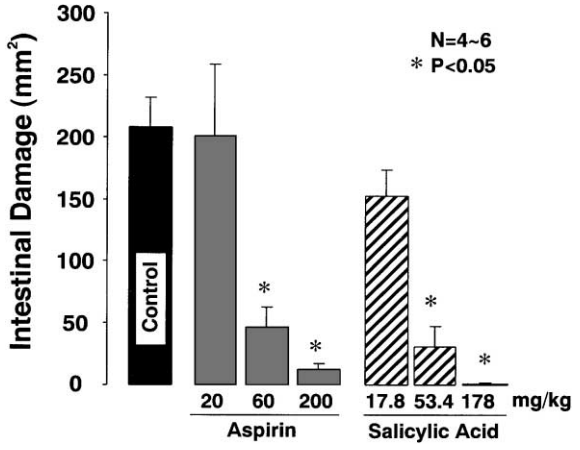

Fig. 3. Effects of aspirin (ASA) and salicylic acid (SA) on indomethacin-induced small intestinal lesions in rats. The animals were given indomethacin (10 mg/kg) s.c., and they were killed 24 h later. ASA (20–200 mg/kg) or SA (17.8–178 mg/kg) were given s.c. 30 min before indomethacin. Data were presented as the means±S.E. from 4 to 6 rats. *Significant difference from control, at $P<0.05$.

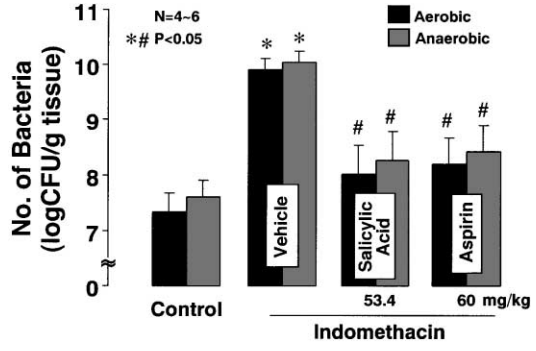

Fig. 4. Effects of aspirin (ASA) and salicylic acid (SA) on the increased enterobacterial translocation caused by indomethacin in rats. Animals were given indomethacin (10 mg/kg) s.c., and the bacterial numbers in the mucosa were counted 24 h later. ASA (60 mg/kg) or SA (53.4 mg/kg) were given s.c. 30 min before administration of indomethacin. Data were presented as the means±S.E. from 4 to 6 rats. *Significant difference from control, at $P<0.05$.

3.3. Effects of ASA and SA on intestinal hypermotility caused by indomethacin

Indomethacin (10 mg/kg) given subcutaneously produced a marked enhancement of intestinal motility, in terms of both the amplitude and frequency of contraction (Fig. 5). The enhanced intestinal motility caused by indomethacin was apparently inhibited by subsequent s.c. administration of ASA (60 mg/kg) or SA (53.4 mg/kg), and the amplitude of contractions gradually decreased to those observed in basal contractions. In addition, this effect of SA was observed much earlier than that of ASA.

3.4. Blood SA levels after administration of ASA

Following s.c. administration of ASA (200 mg/kg) in rats, the plasma levels of SA were increased with time, reaching almost plateau levels within 30 min; the values were 168.2±8.1 μg/ml and remained elevated for more than 7 h (Fig. 6).

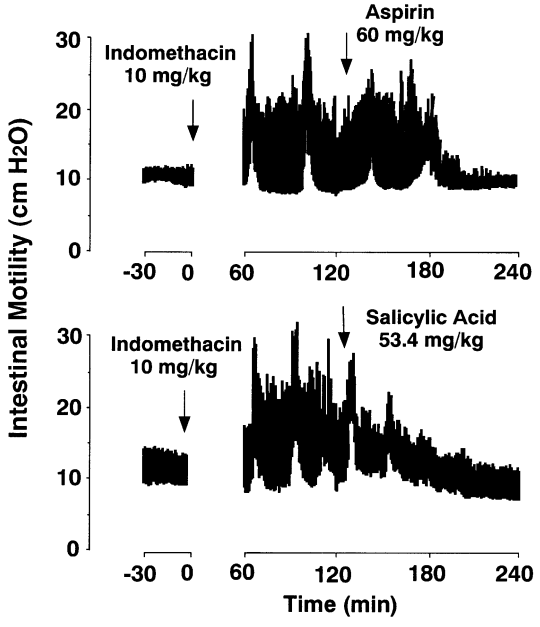

Fig. 5. Representative recordings showing the effects of aspirin (ASA) and salicylic acid (SA) on indomethacin-induced intestinal hypermotility in rats. Animals were given indomethacin (10 mg/kg) s.c., and subsequently 2 h later either ASA (60 mg/kg) or SA (53.4 mg/kg) was given s.c.. Note that both ASA and SA potently inhibited intestinal hypermotility induced by indomethacin, and the effect of SA appeared much earlier than that of ASA.

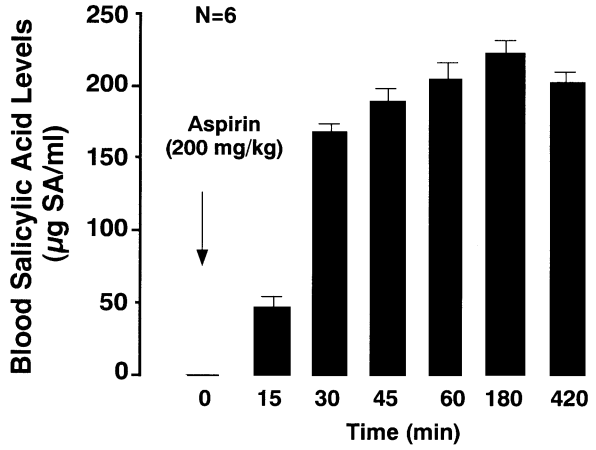

Fig. 6. Plasma salicylic acid (SA) levels after administration of aspirin (ASA) in rats. The animals were given ASA (200 mg/kg) s.c., and the blood samples were collected from a tail vein at various time points after the administration. Data are presented as the means±S.E. from 6 rats per group.

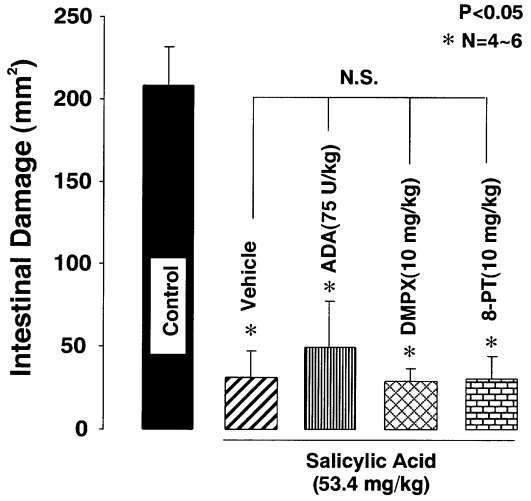

Fig. 7. Effects of adenosine deaminase and adenosine receptor antagonists on the protective action of salicylic acid (SA) against indomethacin-induced small intestinal lesions in rats. Animals were given indomethacin (10 mg/kg) s.c., and they were killed 24 h later. SA (53.4 mg/kg) was given s.c. 30 min before indomethacin treatment, while the adenosine deaminase (ADA: 75 U/kg), 3,7,-dimethyl-1-propargylxanthine (DMPX; 10 mg/kg) or 8-phenyltheophylline (8-PT; 10 mg/kg) was given s.c. 30 min before administration of SA. Data were presented as the means±S.E. from 4 to 6 rats. *Significant difference from control, at $P<0.05$.

3.5. Effects of adenosine deaminase and adenosine receptor antagonists on the protective action of SA against indomethacin-induced intestinal lesions

To investigate the possible involvement of endogenous adenosine in the SA action, we examined the effect of various drugs, affecting the adenosine action, on the protection by SA of indomethacin-induced intestinal damage. Again, indomethacin (10 mg/kg) produced severe lesions in the small intestine within 24 h. These lesions were significantly prevented by prior administration of SA (53.4 mg/kg), the inhibition being 85.4% (Fig. 7). This protective effect of SA was significantly affected by pretreatment of the animals with neither of the agents tested; the adenosine deaminase (75 U/kg, i.p.), 8-PT the adenosine-1 receptor antagonist (10 mg/kg, s.c.) nor DMPX the adenosine-2 receptor antagonist (10 mg/kg, s.c.). The efficacy of SA in reducing the severity of indomethacin-induced intestinal damage was almost the same in any groups, treated with or without the above agents.

4. Discussion

The present study showed that ASA prevented the occurrence of intestinal lesions induced by NSAIDs such as indomethacin. In addition, we also found that the protective action of ASA is shared by SA, the metabolite of ASA, and the protective mechanism may be related, at least partly, with inhibition of intestinal hypermotility caused by indomethacin.

First, we confirmed that conventional NSAIDs provoked damage in the small intestinal mucosa, together with a decrease of mucosal PGE_2 contents and increase of enterobacterial translocation [8,10,14,21,22]. Since ASA was not ulcerogenic in the small intestine, despite decreasing the mucosal PG production as effectively as other NSAIDs, it is unlikely that a depletion of endogenous PGs by itself is not sufficient for induction of small intestinal lesions. The present results also suggest a close relationship between the bacterial translocation and the occurrence of intestinal damage following administration of NSAIDs. However, ASA decreased PGE_2 contents in the intestinal mucosa, yet did not increase the bacterial translocation in the mucosa. These results indicate no direct causal relationship between a PG deficiency and the enterolbacterial translocation, and suggest that other additional factors may be prerequisite for the bacterial translocation following administration of NSAIDs.

On the other hand, ASA did not provoke damage in the small intestine but showed a dose-dependent inhibition against indomethacin-induced intestinal lesions. This result is in agreement with the finding by Robert et al. [15,16]. who showed for the first time that ASA exhibited cytoprotective action against intestinal damage in response to indomethacin. In the present study, ASA decreased the mucosal PGE_2 contents as effectively as other NSAIDs that provoked intestinal lesions, excluding a negative interaction on the PG biosynthetic activity or the pharmacokinetics between ASA and indomethacin [13]. Of interest, this protective effect of ASA was totally mimicked by SA, the action being more potent than that of ASA. Because high blood

levels of SA, the major metabolite of ASA, was observed after administration of ASA, it is possible that the protective action is mediated by SA. Certainly, SA significantly prevented the bacterial translocation which plays a critical role in the development of intestinal lesions following indomethacin.

NSAIDs including indomethacin are known to increase gastric motility, the phenomenon being important in gastric ulcerogenic response to indomethacin [17]. We also found that indomethacin at the intestinal ulcerogenic dose also produced a marked enhancement of intestinal motility, in terms of both the frequency and the amplitude of contractions [11]. Of interest, the enhanced intestinal motility caused by indomethacin was suppressed by ASA as well as SA. It is known that intestinal peristaltic contractions are important in modulating the tissue integrity against luminal pathogens in the gastrointestinal tract. Since hypercontraction of the intestine results in disruption of the unstirred mucus layer over the epithelium, leading to an increase in the mucosal susceptibility to enterobacteria, the intestinal hypermotility may be involved in the pathogenic mechanism of indomethacin-induced small intestinal lesions. Furthermore, since the intestinal hypermotility may cause the mucosal hypoxia and microvascular injury due to smooth muscle contraction, leading to neutrophil infiltration and release of various cytokines [1,12], it is also assumed that these functional changes increase the mucosal susceptibility to initial insults following indomethacin. Indeed, Anthony et al. [1,2] showed the importance of the damaging effect of indomethacin on blood flow to regions of the rat small intestine that are sensitive to ischemia. Thus, the inhibition of intestinal motility may account at least partly for the protection by ASA or SA of indomethacin-induced small intestinal lesions. At present, the mechanism how indomethacin enhanced intestinal motility and how SA suppressed the intestinal hypermotility induced by indomethacin remains unknown. Yet, because indomethacin-induced gastric hypermotility is mediated by vagal-cholinergic mechanism [17], the same might be applied to the intestinal hypermotility.

It has been recently shown that salicylates increase ATP hydrolysis and thereby enhance release of adenosine, and that the anti-inflammatory action of SA is partly mediated by endogenous adenosine [5]. The same authors also showed that removal of adenosine by adenosine deaminase or specific antagonism of adenosine at A2 receptors completely reversed the anti-inflammatory effects of aspirin and sodium salicylate [6,7]. So, it is possible that the protective effect of SA is also due to the action of adenosine. In the present study, however, we observed that neither adenosine deaminase, 8-PT (A1 antagonist) nor DMPX (A2 antagonist) had any influence on the protective action of SA on indomethacin-induced intestinal lesions. Thus, it is unlikely that the intestinal protective action of ASA or SA is mediated by endogenous adenosine.

In conclusion, the present results taken together suggest that SA has a cytoprotective action against indomethacin-induced small intestinal lesions, and this action may be associated with inhibition of the intestinal hypermotility and the bacterial translocation, but not mediated by endogenous adenosine. Failure of ASA to induce intestinal damage may be explained, at least partly, by a protective action of SA, the metabolite of ASA. Further studies should be needed to explore the exact mechanism by which these drugs protect the small intestine against the ulcerogenic action of indomethacin.

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