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Salicylic Acid Blocks Indomethacin-Induced Cyclooxygenase Inhibition and Lesion Formation in Rat Gastric Mucosa (42007)

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Abstract. Salicylic acid has been shown to decrease gastric mucosal lesions induced by indomethacin in the rat. *In vitro,* it has also been shown to counteract the inhibitory effect of indomethacin and aspirin on the cyclooxygenase enzyme system in seminal vesicle microsomes and in platelets and vascular tissue. The hypothesis that the mechanism of salicylic acid "protection" against indomethacin-induced gastric lesions involves interference with indomethacin-induced mucosal cyclooxygenase inhibition was tested. Male, fasted rats were treated with intragastric salicylic acid in doses of 50, 100, 200, 300, or 400 mg/kg concomitantly with a sc injection of 20 mg/kg of indomethacin. Gastric mucosal lesions and mucosal cyclooxygenase activity (as measured by *ex vivo* prostaglandin $F_{2\alpha}$ synthesis) were examined 3 hr later. Intragastric salicylic acid, 200–400 mg/kg, significantly reduced indomethacin-induced lesion formation, while counteracting significantly indomethacin inhibition of prostaglandin synthesis. Salicylic acid alone did not significantly change cyclooxygenase activity. It is concluded that topical salicylic acid can decrease indomethacin-induced gastric mucosal lesion in the rat, in part, by counteracting the inhibitory effect of indomethacin at the cyclooxygenase level.

Nonsteroidal anti-inflammatory drugs (NSAID) such as aspirin or indomethacin have been shown to damage the gastric mucosa in animals (1, 2) and in human subjects (3). These drugs also inhibit cyclooxygenase, a key enzyme in the prostaglandin (PG)-forming system (4, 5), and this may be the mechanism of their damaging effect (6). Salicylic acid, which has no inhibitory effect on gastric mucosal cyclooxygenase *in vivo* (7), has been shown to reduce indomethacin-induced gastric mucosal lesions in the rat (8, 9), as well as its derivative, diflunisal (10). Recently it has been reported that salicylic acid can block the inhibitory effect of indomethacin and aspirin on the cyclooxygenase system in microsomes (11) and platelets (12–14). The present study was designed to examine simultaneously the effect of salicylic acid on indomethacin-induced damage and cyclooxygenase inhibition in the rat gastric mucosa.

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Materials and Methods. Male Sprague–Dawley rats (180–210 g) were fasted for 24 hr, and 12 hrs prior to the experiment were placed in individual cylindrical metal cages to avoid coprophagy (15).

Materials. A suspension of salicylic acid (Sigma, St. Louis) in distilled water was freshly prepared before each experiment. Tween 80 (Sigma), 2 drops/20 ml, was added and the suspension was shaken for 60 min prior to treatment. Indomethacin (Sigma) was dissolved in 5% sodium bicarbonate, then further diluted with water (1:5) to 1% bicarbonate solution.

Methods. Two studies were performed. A randomized design was used in each. In both, 1 ml salicylic acid was administered intragastrically (ig) concomitantly with a sc injection of a single fixed dose of indomethacin (20 mg/kg). In study 1, salicylic acid was given in the following doses: 50, 100, and 200 mg/kg. In study 2, the doses were 200, 300, and 400 mg/kg. The appropriate controls were treated with indomethacin, salicylic acid, or vehicle. Three hours after treatment, the stomachs were opened along the greater curvature, and each lesion in the glandular part

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was scored as follows by an observer who was unaware of the treatment group to which the rats belonged: petechial lesion = 1; erosion less than 1 mm = 2; erosion between 1 and 2 mm = 3; erosion between 2 and 4 mm = 4; erosion greater than 4 mm = 5. The scores for each lesion were then summed to obtain the final score for the given stomach. Immediately after scoring, the corpus mucosa was stripped, weighed (145–155 mg), chopped, and washed in phosphate buffer, pH 7.4. PG generation was performed by vortexing the tissue for 1 min at room temperature (7). $PGF_{2\alpha}$, a stable end product of the cyclooxygenase system, was determined in the supernatant of each sample by a specific radioimmunoassay (RIA) described earlier (16). The capacity of the tissue to synthesize PG is expressed as nanogram per gram wet weight. The interassay variation in this study was 5–10%. Among the various groups of rats, we observed variability in the sensitivity to lesion formation by indomethacin. Studies 1 and 2 were performed on separate groups of rats. Rats for each study were obtained at different times from the same supplier (Simonsen Labs). Rats in study 2 were more sensitive to indomethacin-induced lesion formation (see Figs. 1 and 2). The small standard errors, however, indicate that rats within each study were homogeneous in this regard.

Statistical analysis. The results of each experiment were analyzed using two-way analysis of variance (factors were indomethacin, no indomethacin and dose of salicylic acid) followed by tests of specific contrasts. The significance level was two-sided $P < 0.05$ throughout.

Results. *Study 1.* The effect of 50–200 mg/kg salicylic acid ig on indomethacin-induced inhibition of prostaglandin synthesis and lesion formation (Fig. I): Salicylic acid did not prevent indomethacin-induced lesions in this dose range. However, PG generation in the 200 mg/kg salicylic acid + indomethacin group was significantly higher than that of the indomethacin alone group. Further examination suggested that this group consisted of two distinct subgroups. The one (n = 5) showed a PG synthesis rate of 71 ± 7 ng/g wet wt and a lesion score of 2.2 ± 0.9, while a second group (n = 6) with a much lower

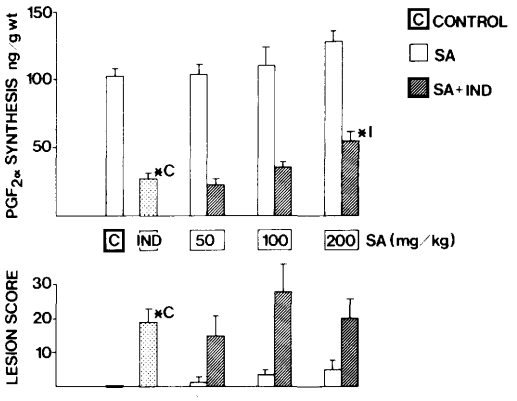

FIG. 1. Effect of intragastric salicylic acid (50–200 mg/kg) on indomethacin-induced mucosal lesions and cyclooxygenase activity (mean ± SE). Groups of rats (n = 10 except from the 200 mg/kg group n = 11) were randomly administered either vehicle or SA, 50, 100, or 200 mg/kg in 1 ml, simultaneously with a subcutaneous injection of IND, 20 mg/kg in 1 ml. Three hours later, gastric mucosal lesions were scored and cyclooxygenase activity was determined by *ex vivo* PG generation. $PGF_{2\alpha}$ was determined by a specific radioimmunoassay and expressed in ng/g wet tissue. IND significantly inhibited PG synthesis and produced gastric erosions as compared with C (*C, $P < 0.05$). SA, 200 mg/kg, significantly decreased the IND inhibition of PG synthesis (*I, $P < 0.05$).

PG synthesis value, 36 ± 5, had a much higher lesion score, 44 ± 5. This constituted the basis for study 2 in which higher doses of salicylic acid were applied.

Study 2. The effect of 200–400 mg/kg salicylic ig on indomethacin-induced inhibition of prostaglandin synthesis and lesion formation (Fig. 2): In this study 200 mg/kg salicylic ig significantly reduced indomethacin lesions from 32 ± 7 to 15 ± 6 ($P < 0.05$). The higher doses, 300 and 400 mg/kg, showed even greater "protection." In addition, administration of 200–400 mg/kg salicylic acid ig to the indomethacin-treated rats significantly prevented the decrease in mucosal PG synthesis. In these groups PG synthesis was 63 to 77% of normal, compared with 18% for indomethacin alone. Lesions were observed with these high doses of salicylic acid ig alone, but were smaller and milder in severity than indomethacin lesions.

Discussion. Nonsteroidal anti-inflammatory drugs such as aspirin and indomethacin were found to be inhibitors of the PG syn-

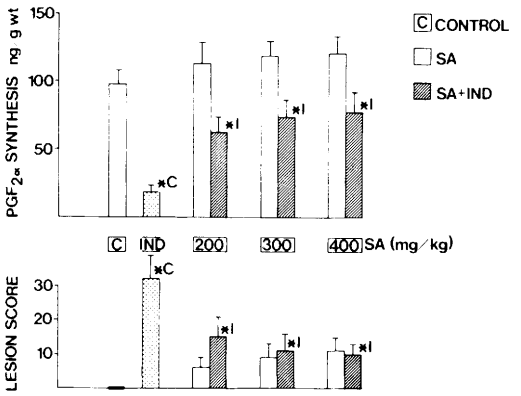

FIG. 2. Effect of intragastric salicylic acid (200–400 mg/kg) on indomethacin-induced mucosal lesions and cyclooxygenase activity (mean ± SE). Groups of rats (n = 10) were randomly treated with either vehicle or SA, 200, 300, or 400 mg/kg in 1 ml, simultaneously with a subcutaneous injection of IND, 20 mg/kg in 1 ml. Three hours later, gastric mucosal lesions were scored and cyclooxygenase activity was determined as described in legend to Fig. 1. IND significantly inhibited PG synthesis and produced gastric erosions as compared with C (*C, $P < 0.05$). PG synthesis in all SA + IND-treated groups was significantly higher than in the IND-treated group (*I, $P < 0.05$). SA significantly reduced lesions as compared with rats treated with IND alone (*I, $P < 0.05$).

thetase system, and this was postulated to be the mechanism of their action (4). These drugs have also been found to produce gastrointestinal damage in animals and human subjects (1–3, 5, 19, 20). The damage induced by NSAID and other injurious agents is inhibited by exogenous PG (7, 18) indicating that inhibition of PG synthesis predisposes to gastrointestinal damage. Salicylic acid, a weak cyclooxygenase inhibitor *in vitro* (4) but not in the gastric mucosa (5, 16), has been found to block indomethacin-induced mucosal lesions in the rat (8, 9) and to protect against ethanol-induced damage (18). *In vitro* studies (11–14, 17) have suggested that salicylic acid might interfere with indomethacin or other NSAID action at the cyclooxygenase level.

Our present study shows that, in the rat, ig salicylic acid counteracts the ulcerogenic effect of indomethacin and partially blocks its inhibitory effect on gastric mucosal PG synthesis as measured by *ex vivo* $PGF_{2\alpha}$ generation. This effect was observed with 200–400 mg/kg of salicylic acid, a slightly higher dose range than that reported by Humes *et al.* for enzyme interaction (11) and by Robert for antiulcer effects (18). In the present study, however, indomethacin was administered sc while salicylic acid was given ig to avoid the possibility of local interference between the drugs. This *in vivo* study confirms and extends previous *in vitro* observations that salicylate reduces indomethacin-induced gastric mucosal cyclooxygenase inhibition (11). The present and previous studies (5, 16) did not reveal any inhibitory effect of salicylic acid on mucosal cyclooxygenase. This does not confirm the hypothesis that salicylic acid as a mild irritant induces "adaptive cytoprotection" by enhancing mucosal PG synthesis (18). Salicylic acid by itself produced mucosal lesions which were, however, mild and clearly differed from the indomethacin lesions which were usually elongated and deeper.

The mechanism by which salicylic acid blocks indomethacin-induced mucosal cyclooxygenase inhibition is unknown. It may partially be due to direct interference between the drugs, thus preventing the indomethacin from exerting its maximal action (9), although unlikely in this study since the drugs were administered via different routes. Humes *et al.* (11) believe the interaction between salicylic acid and indomethacin occurs at the cyclooxygenase enzyme level. They have postulated the existence of the following binding sites on the enzyme for NSAID: a catalytic site which determines the inhibitory potency of the drug and a supplementary site which is also essential for this inhibition. Drugs such as salicylic acid bind more effectively to the latter binding site, thus preventing the inhibitory effect of the potent NSAID. The results of our study are consistent with this theory. However, since salicylate "protects" not only against cyclooxygenase inhibitors but also against other injurious agents such as ethanol (18) and since significant PG inhibition does not necessarily accompany lesion formation (23), salicylate protection against NSAID-induced lesions may only partially be due to its effect on the cyclooxygenase in the gastric mucosa.

In summary, our study indicates that salicylic acid protects against indomethacin-

induced mucosal lesions simultaneously with partial but significant blocking of the inhibitory effect of indomethacin on PG synthesis. The latter may explain, in part, the protective effect of salicylic acid against indomethacin and other NSAID-induced gastric mucosal lesions.

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