CORRELATION BETWEEN THE STIMULATION OF PROSTAGLANDIN BIOSYNTHESIS BY HYDROXYPHENYL ALKANOIC ACIDS AND THEIR ANTI-ULCEROGENIC ACTIVITY

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It is well-known that prostaglandin (PG) synthetase inhibitors such as aspirin and indomethacin induce gastrointestinal ulcer both in experimental animals and humans. In addition, it has been reported that PG levels in the plasma and gastric juice of patients with ulcer are significantly lower than these levels in healthy subjects (1). On the other hand, PGE type PGs and their analogs are effective against experimental ulcer produced in animals (2-6). These results suggest that stimulants of PG synthetase may prevent ulceration in the gastrointestinal mucosa and repair an already formed ulcer. We attempted to determine whether the stimulation of PG biosynthesis *in vitro* by hydroxyphenyl alkanolic acids correlates with anti-ulcerogenic activity of the acids in the rat.

PG synthetase activity was assayed according to the method of Tachizawa *et al.* (7) in the following incubation mixture at 37°C for 30 min: 7.5 mg of lyophilized microsomes of the rabbit renal medulla, 30 μ M 3 H-arachidonic acid, 1.7 mM reduced *l*-glutathione, 0.07 mM EDTA-2Na and 1 mM hydroxyphenyl alkanolic acids or reference compound, 0.5 mM *l*-epinephrine bitartrate, in 1.5 ml of 0.2 M Tris-HCl buffer (pH 7.5). PGs formed from the substrate were extracted and separated by TLC and the radioactivity of each spot on the TLC plate was measured by a liquid scintillation counter. Percent increase was obtained by comparing the percentage of radioactivity converted from the labeled substrate into PGE₂ with or without hydroxyphenyl alkanolic acids or *l*-epinephrine.

Anti-ulcerogenic activity of hydroxyphenyl alkanolic acids was expressed as percent inhibition against aspirin-induced gastric ulcer. Seven male Sprague-Dawley rats weighing 106 to 148 g, (Charles River), were fasted overnight. Aspirin was administered p.o. to the animals at a dose level of 200 mg/kg in a suspension of 0.5% carboxymethyl cellulose solution and 3.5 hr later the animals were sacrificed. The stomachs were removed and examined macroscopically for gastric ulcer. Ulcer index was expressed as sum of the length of each lesion. Test compounds (250 mg/kg) were given p.o. in a suspension of 0.5% carboxymethyl cellulose solution 1 hr before the aspirin administration. Compounds tested were as follows: *p*-hydroxybenzoic acid (I), 5-hydroxypyridine-2-carboxylic acid (II), 3,4,5-trihydroxybenzoic acid (III), *p*-hydroxyphenylacetic acid (IV), 3-(*p*-hydroxyphenyl)propionic acid (V), 3-(*m*-hydroxyphenyl)propionic acid (VI), 3-(*o*-hydroxyphenyl)propionic acid (VII), phenylpropionic acid (VIII), 3-(*p*-methoxyphenyl)propionic acid (IX), 3-(3,4-dihydroxyphenyl)propionic acid (X), 4-(*p*-hydroxyphenyl)butyric acid (XI), 6-(*p*-hydroxyphenyl)hexanoic acid (XII), 3-(*p*-hydroxyphenoxy)propionic acid (XIII).

As shown in Table 1, PGE₂ formation rate in control was 9.4% and the addition of 0.5 mM *l*-epinephrine bitartrate to control increased the rate to 25.3%. When hydroxyphenyl alkanolic acids were added to control at a final concentration of 1 mM, compounds I, III-VII, X, XI and XIII having hydroxyphenyl group in their structure indicated remarkable stimulative effects on PGE₂ formation. These effects were dependent on the increase in the number of hydroxy group (III, X), but not on the position of substitution of the group (VI, VII). On the contrary, compounds VIII and IX lacking the hydroxy group or having the methoxy group instead of the hydroxy showed no stimulative effects. In addition, the effects were remarkably reduced by increasing the number of methylene group of the acids (XI, XII) or substituting the pyridinyl group for the phenyl one in the structure of the acids (II). Fig. 1 shows the comparison between the stimulative effect of PG biosynthesis by five compounds (I, III, IV, V, X) and that by *l*-epinephrine. Compound X was as potent as *l*-epinephrine and compounds III and V showed approximately 0.5 times the stimulative potency of *l*-epinephrine. As shown in Table 1, of nine compounds tested, I, IV, V and X exhibited potent anti-ulcerogenic effect as well as a definite stimulative effect on PG synthesis, while compounds II and XII showed weak effects. Compounds VIII and IX showed neither effect.

Our present results show that hydroxyphenyl alkanolic acids act as cofactors for PG synthetase like *l*-epinephrine. In this connection, Collier *et al.* (8) reported that emetics, purgatives and irritants which facilitate intestinal function and act as cofactors for PG synthetase, have the hydroxyphenyl group. In addition, Baumann *et al.* (9) reported that

TABLE 1. Effect of hydroxyphenyl alkanolic acid on prostagandin biosynthesis *in vitro* and aspirin-induced gastric ulcer in rats

Treatment	PGE ₂ biosynthesis ^{a)}		Aspirin-induced ulcer ^{b)}	
	% formation	% increase	ulcer index	% inhibition
Control	9.4±0.71 ^{b)}		75.9±5.58 ^{b)}	
Compound I ^{c)}	19.9±0.60*	111 ^{c)}	19.4±2.84*	74 ^{b)}
II	11.4±0.68	21	51.1±5.28*	33
III	25.8±0.47*	174	68.1±8.13	10
IV	20.3±1.16*	116	21.3±7.18*	72
V	17.4±0.68*	85	31.8±8.95*	58
VI	18.3±0.46*	95		
VII	17.2±1.05*	83		
VIII	8.1±0.53	-13	61.9±2.72	19
IX	9.3±0.25	1	67.0±5.31	12
X	25.3±1.56*	169	30.8±6.73*	60
XI	16.2±0.80*	73		
XII	9.6±1.15	2	55.3±3.00*	27
XIII	19.7±1.41*	110		

a) Described in Text. b) Values represent the means±standard error. c) Values represent % increase to control. d) Values represent % inhibition to control. Asterisk indicates a significant difference from control (*P<0.01). e) PGE₂ formation rate in *l*-epinephrine bitartrate was 25.3% at a final concentration of 0.5 mM.

FIG. 1. Stimulative effects of hydroxyphenyl alkanolic acids on prostaglandin biosynthesis *in vitro*. I, III, IV, V and X: described in Text. Epinephrine: *l*-epinephrine bitartrate.

3-(3,4-dihydroxyphenyl)propionic acid (X) stimulates PG synthetase in the rat renal medulla, whereas 3-(*p*-hydroxyphenyl)propionic acid (V) at a concentration of 1 mM has little effect. In the present work, 1 mM 3-(*p*-hydroxyphenyl)propionic acid (V) showed a half stimulative effect of 0.5 mM *l*-epinephrine on PG synthetase in the rabbit renal medulla. The discrepancy between the results may be explained by the findings that the drug sensitivity of PG synthetase in the rabbit is remarkably higher than that in the rat (7). Moreover, our present results demonstrate that most hydroxyphenyl alkanolic acids have the ability to stimulate PG synthetase and to repair lesions in case of aspirin-induced ulcer.

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