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EFFECT OF 2′-CARBOXYMETHOXY-4,4′-BIS(3-METHYL-2-BUTENYLOXY)CHALCONE (SU-88) ON PROSTAGLANDIN METABOLISM IN HOG GASTRIC MUCOSA

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Abstract—SU-88 [2′-carboxymethoxy-4,4′-bis(3-methyl-2-butenyloxy)chalcone] inhibited the activity of the prostaglandin (PG)-metabolizing enzyme, 15-hydroxy-PG-dehydrogenase (15-OH-PG-DH), in a cytoplasmic fraction of gastric mucosa. This compound had no effect on the PG synthetase of bovine seminal vesicle microsomes and lactate dehydrogenase in rat liver. The 15-OH-PG-DH activity of gastric mucosa was not influenced by a specific inhibitor of alcohol dehydrogenase, 4-methylpyrazole. Carbenoxolone (CBX) also inhibited 15-OH-PG-DH activity. The IC_{50} values for SU-88 and CBX were approximately 20 and 40 μM respectively. SU-88 inhibited 15-OH-PG-DH activity uncompetitively or competitively according to whether PGE_1 or NAD was used as substrate. CBX inhibited competitively the activity of this enzyme for both substrates. After the addition of SU-88 or CBX to the incubation medium of gastric mucosa, the PGE_2 level of the medium was increased significantly while that of the tissue remained unchanged. These results indicate that SU-88 specifically inhibited 15-OH-PG-DH activity and suggest that 15-OH-PG-DH activity regulates the level of PGs in gastric mucosa and may have an anti-ulcer influence.

Certain prostaglandins (PGs) inhibited the gastric acid secretion stimulated by feeding, histamine, or gastrin [1, 2]. These PGs are vasodilators in the gastric mucosa and may be involved in the local regulation of blood flow [1]. They prevent ulcer formation and accelerate ulcer healing [3–7]. It has been considered that the anti-ulcer effects of PGs are due to the inhibition of gastric acid secretion and/or the vasolidation in gastric mucosa. Recently, protective effects of PGs have been observed on gastric lesions formed by a variety of necrotizing agents, without reducing gastric secretion [8]. In fact, synthesis of PGE_2 and PGI_2 has been observed in gastric mucosal cells [9, 10]. PGs have anti-ulcer and cytoprotective effects on gastric mucosa.

[2′-Carboxymethoxy-4,4′-bis(3-methyl-2-butenyloxy)chalcone (SU-88) is a new anti-ulcer compound that was recently developed [11–13] (Fig. 1). It has been found to dilate blood vessels in the gastric mucosa and to increase local gastric blood flow [14]. Furthermore, this compound stimulates gastric mucus production and enhances the incorporation of ^{35}S into gastric mucosal sulfated mucosubstances in restrained and water-immersed rats [15]. Like humans, hogs produce more gastric mucosal PGE_2 relative to 6-keto-$PGF_{1\alpha}$ and respond to stress and nonsteroidal anti-inflammatory drugs with gastric ulceration [16–18]. It has also been reported that the morphology of the gastrointestinal tract and gastric function in the hog are basically the same as in humans [17]. In this study, we have examined how PGs may contribute to the anti-ulceric effects of SU-88 using hog mucosal tissues.

MATERIALS AND METHODS

Enzyme preparation from hog gastric mucosa and guinea pig lungs. Fresh hog stomachs were obtained from a local slaughterhouse. After the stomachs were washed in tap water and 0.9% NaCl, the fundic mucosa was scraped from the underlying muscular layer. The mucosa was homogenized with 4 vol. of an ice-cold 150 mM potassium phosphate buffer (pH 7.4) containing 2 mM EDTA, 2 mM mercaptoethanol, 300 μM NAD and 200 mg/l bovine serum albumin by Ultra-Turrax (Janke & Kunkel) for 10 sec. Following centrifugation at 100,000 *g* for 60 min, the resultant supernatant fraction was used as the enzyme source of the gastric mucosa. The enzyme preparation of guinea pig lungs was obtained by the same procedure as described above, after perfusion of the lung via the inferior vena cava with 20 ml of ice-cold 0.9% NaCl.

Assay of 15-hydroxy-PG-dehydrogenase (15-OH-PG-DH) activity. The activity of 15-OH-PG-DH was determined as previously reported [19, 20]. As a substrate, PGE_1 was incubated with enzyme preparations from hog gastric mucosa and guinea pig lung. The reaction mixture was contained in a total volume of 2.0 ml, the same buffer as that used for the homogenization, enzyme preparation, and PGE_1. PGE_1 (50 μM) and NAD (300 μM) were used as

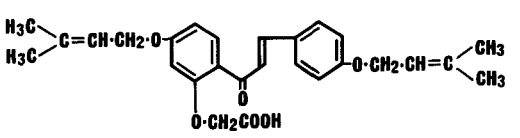

Fig. 1. Chemical structure of 2′-carboxymethoxy-4,4′-bis(3-methyl-2-butenyloxy)chalcone (SU-88).

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substrates unless otherwise mentioned. The reaction was initiated by the addition of PGE_1. Incubation was done at 37° and was terminated by the addition of 0.5 ml of 2 N NaOH. The maximum absorbance at 500 nm was measured about 2 min later using a spectrophotometer (Shimazu UV 240). The product, 15-keto-PGE_1, was quantitated from the extinction coefficient (30,300 M^{-1}/cm) of the chromophore generated in the base.

Assay of the activities of hepatic lactate dehydrogenase (LDH) and PG synthetase of bovine seminal vesicle microsomes (BSVM). LDH activity was determined as previously reported [21]. The supernatant fraction obtained by centrifugation of rat liver homogenate at 100,000 *g* for 30 min was used as the enzyme preparation. The reaction was carried out in 3.0 ml of a mixture consisting of pyruvic acid (10 mM), NADH (250 μM), enzyme (200 μg) and pH 8.0 potassium phosphate buffer (70 mM); it was started by adding enzyme and was measured as the decrease in the absorbance at 340 nm after 4 min.

The activity of PG synthetase was measured by a slight modification of the method previously reported [22]. The microsomal fraction of bovine seminal vesicle PG synthetase was obtained from Miles Laboratories. PG biosynthesis was carried out in 0.5 ml of a reaction mixture consisting of [1-^{14}C]arachidonic acid (0.38 μM, sp. act. 52.9 mCi/mmole, New England Nuclear Corp.), enzyme (100 μg protein), hematin (1 μM), reduced glutathione (1 mM), tryptophan (1 mM) and pH 7.4 potassium phosphate buffer (50 mM). The reaction mixture was incubated at 30° for 2 min; the reaction was stopped by adding 2 ml of *n*-hexane–ethyl acetate (2:1). The remaining arachidonic acid in the reaction medium was extracted to the organic phase by mixing with *n*-hexane–ethyl acetate, according to the method of Yanagi and Komatsu [22]. Since PGE_2 selectively remained in the aqueous phase, the PG biosynthesis activity was determined by measuring the radioactivity in the aqueous phase with a liquid scintillation spectrometer (Packard TriCarb model 3375). Under these reaction conditions, PGE_2 was the main product and made up more than 93% of the total product. By this selective extraction more than 90% of the PGE_2 was recovered from the reaction medium.

Extraction and identification of PGE_2 from hog gastric mucosa and reaction medium. After being washed with a modified Krebs–Ringer Tris buffer consisting of 118 mM NaCl, 4.7 mM KCl, 2.5 mM KH_2PO_4, 1.2 mM $MgSO_4$, 17.5 mM Tris–HCl (pH 7.4), 1 mM glucose and 100 mg/dl of bovine serum albumin, approximately 200 mg of gastric mucosa was preincubated in 2.0 ml of the modified Krebs–Ringer Tris buffer for 10 min at 37°. After discarding the preincubation medium, the tissues were incubated in 2.0 ml of the modified Krebs–Ringer Tris buffer for 60 min at 37° in the presence or absence of the anti-ulcer compounds.

Mucosal tissues were separated from the medium 60 min later. The PGs were extracted from the medium by mixing the medium with 4 ml of ethyl acetate and 60 μl of 1 N formic acid. The tissues were homogenized after standing with 4 ml of ice-cold ethanol for a few minutes, and, then, filtered. The filtrate was evaporated with the Savant Concentrator under reduced pressure. The residues were dissolved in 0.5 ml of water, and the PGs were extracted from the water to 2 ml of ethyl acetate as described above. Prior to the extraction of PGs from the reaction medium and tissue homogenate, 9000 dpm of [^{3}H] PGE_2 (New England Nuclear, sp. act. 165 Ci/mmole) were added to the medium and the homogenate to determine the recovery of PGE_2. The ethyl acetate containing the PGs extracted from the medium and the tissue was evaporated with the Savant Concentrator under reduced pressure. The resultant residues were dissolved in 100 μl of methanol–ethyl acetate (1:1), and half of this solution was spotted on a thin-layer chromatographic (TLC) plate of silica gel (Kieselgel 60). The chromatogram was developed with an organic phase of ethyl acetate–iso-octane–acetic acid–H_2O (11:5:2:10) to separate PGE_2 from the other PGs and other substances. The authentic PGE_2 on the plate was identified by spraying the plate with 10% phosphomolybdic acid in ethanol and heating. The portion of the chromatogram having the same R_f value as the authentic PGE_2 in the sample line was scraped off, and PGE_2 was extracted with ethyl acetate from the silica gel. Again, the ethyl acetate was evaporated with the Savant Concentrator under reduced pressure. The residues were dissolved in 1.0 ml of Tris–HCl buffer containing 1 mg gelatin for radioimmunoassay of PGE_2.

Determination of PGE_2. PGE_2 was determined using a radioimmunoassay kit (Clinical Assays). The recoveries of added [^{3}H]PGE_2 from the medium and the tissue homogenates were approximately 70 and 62% respectively. Protein concentration was determined by the method of Lowry *et al.* [23] using bovine serum albumin as the standard.

Drugs. Gefarnate, cetraxate and CBX were prepared from commercial medicines. SU-88 was prepared at our laboratory.

RESULTS

Effects of SU-88 on 15-OH-PG-DH activity. SU-88 inhibited the 15-OH-PG-DH from gastric mucosa in a dose-dependent manner (Fig. 2). Carbenoxolone

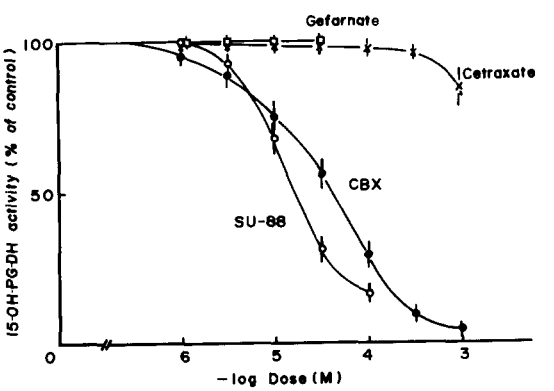

Fig. 2. Inhibition of 15-OH-PG-DH activity by various anti-ulcer drugs. Each point represents the mean of three separate experiments. Control activity was 0.39 ± 0.03 nmoles per mg protein per min.

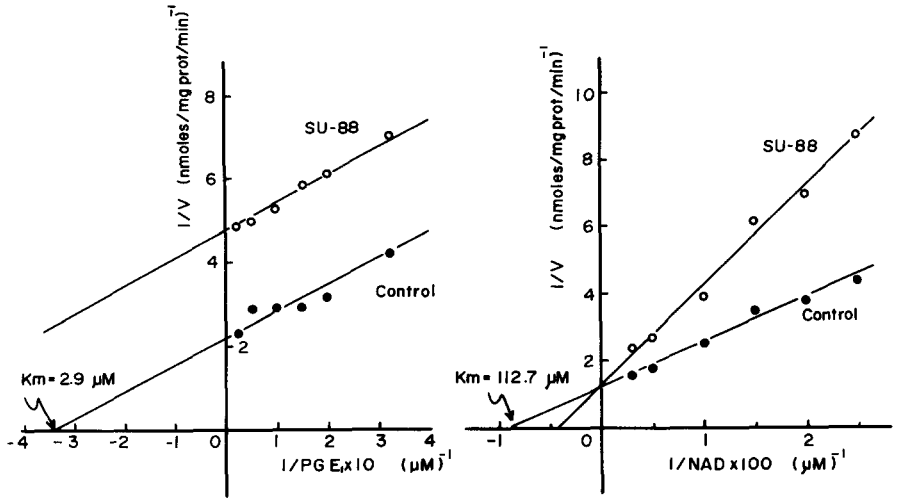

Fig. 3. Double-reciprocal plots of the inhibition of 15-OH-PG-DH activity by SU-88 in hog gastric mucosa. Each point represents the mean of duplicate experiments. A 50 μM concentration of SU-88 was used. Concentrations of PGE_1 and NAD were 3–40 and 40–300 μM respectively. Each point represents the mean of duplicate experiments.

(CBX) also inhibited this enzyme activity, as was reported previously [24, 25], but the other anti-ulcer drugs, cetraxate and gefarnate, had no effect (Fig. 2). The IC_{50} values of SU-88 and CBX were 18 ± 3 and 39 ± 5 μM respectively. SU-88 did not have a significant effect on the PG synthetase obtained from the bovine seminal vesicle microsomes or on the lactate dehydrogenase from rat liver at 10^{-7}–10^{-4} M. Furthermore, 50 μM 4-methylpyrazole, a specific inhibitor of alcohol dehydrogenase, had no effect on the activity of 15-OH-PG-DH from the hog gastric mucosa. The activity of enzyme from guinea pig lung was approximately six times greater than from the mucosa. As with hog gastric mucosa, SU-88 inhibited 15-OH-PG-DH activity of guinea pig lung. The IC_{50} value was 20 ± 3 μM. These results indicate the specificity of SU-88 as an inhibitor of 15-OH-PG-DH.

Kinetic analysis of the inhibitory effect of SU-88 on 15-OH-PG-DH. SU-88 inhibited 15-OH-PG-DH activity uncompetitively when PGE_1 was used as substrate but competitively when NAD was substrate (Fig. 3). The K_i values of SU-88 for PGE_1 and NAD were 32.2 and 32.8 μM respectively. On the other hand, CBX inhibited 15-OH-PG-DH activity of mucosa competitively with either substrate (Fig. 4). The K_i values of CBX for PGE_1 and NAD were 9.7 and 40.5 μM respectively. The same pattern of inhibition by SU-88 of 15-OH-PG-DH activity with PGE_1 as substrate was observed in lung and gastric mucosa (Fig. 5). The K_i value of SU-88 for PGE_1 was 19.5 μM.

Effect of SU-88 on PGE_2 metabolism in hog gastric mucosal tissue. PGE_2 has been considered to play an important role in gastric acid secretion, mucus

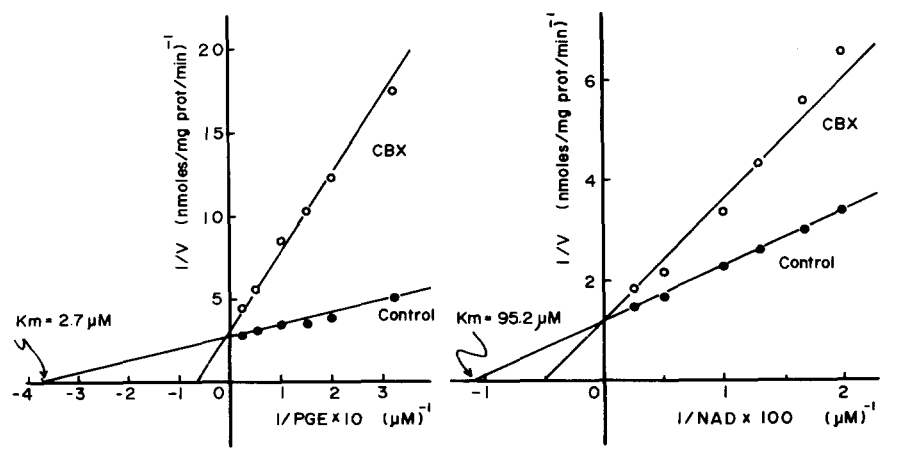

Fig. 4. Double-reciprocal plots of the inhibition of 15-OH-PG-DH activity by CBX in hog gastric mucosa. A 50 μM concentration of CBX was used. Concentrations of PGE_1 and NAD were 3–40 and 40–300 μM respectively. Each point represents the mean of duplicate experiments.

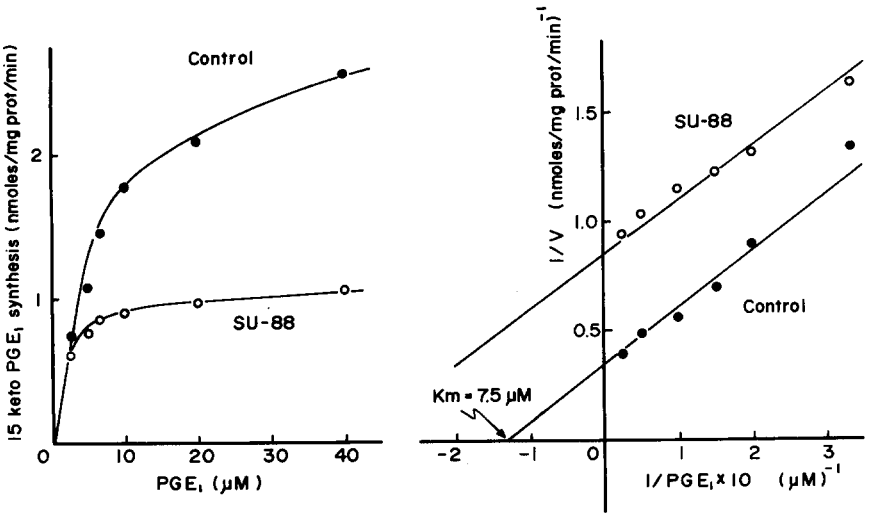

Fig. 5. Inhibition of the activity of 15-OH-PG-DH from the lungs of guinea pigs. A 40 μM concentration of SU-88 was used. Concentrations of PGE_1 ranged from 3 to 400 μM. Each point represents the mean of duplicate experiments.

production and cytoprotection [1–10]. After incubating the hog gastric mucosa with SU-88, a significantly large amount of PGE_2 was obtained in the reaction medium compared with that found in the absence of SU-88 (Fig. 6). The level of PGE_2 remaining in the tissue was not changed by the presence of SU-88 except when treated with 100 μM SU-88. The total amount of PGE_2 in the incubation medium plus tissue was increased significantly by SU-88. Similarly, the amount of PGE_2 in the incubation medium increased significantly, but the level in the tissue was not changed by the presence of 50 μM CBX (Fig. 7). A considerable variation in the PGE_2 level of hog gastric mucosa was observed, due probably to the peculiarities of each hog mucosa, such as age of the hog and the time at which the experiment was carried out. Clarification of this will require further research.

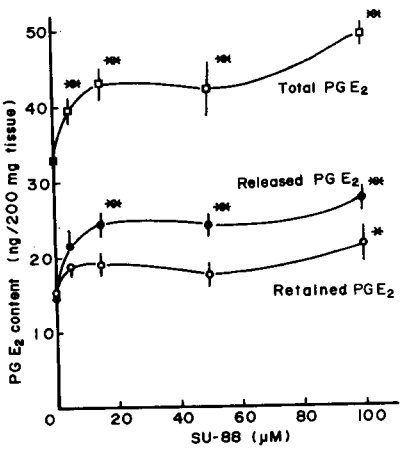

Fig. 6. Effect of SU-88 on PGE_2 levels in tissue (retained) and medium (released). Each point represents the mean ± S.E.M. of four experiments. Key: (*) $P < 0.05$ and (**) $P < 0.01$, compared with each control value respectively.

DISCUSSION

The present study demonstrates that the anti-ulcer compound SU-88 inhibits the activity of the PG-metabolizing enzyme 15-OH-PG-DH; the inhibition patterns for the substrates PG and NAD were uncompetitive and competitive respectively. CBX, an anti-ulcer drug, also inhibited the 15-OH-PG-DH activity, but CBX inhibited the enzyme activity competitively for both PG and NAD. SU-88 inhibited 15-OH-PG-DH activity both competitively and uncompetitively, depending on the substrate. The mechanism of competitive inhibition with NAD as substrate is well understood, whereas the mechanism of uncompetitive inhibition is not clear yet. In general, however, an uncompetitive inhibitor that inhibits two-substrate reactions such as dehydrogenase reactions decreases the rate of conversion from enzyme–substrate complex to enzyme and production by complex formation of enzyme–substrate–inhibitor [26]. Thus, SU-88 probably inhibits the change from the 15-OH-PG-DH-PG complex to 15-OH-PG-DH

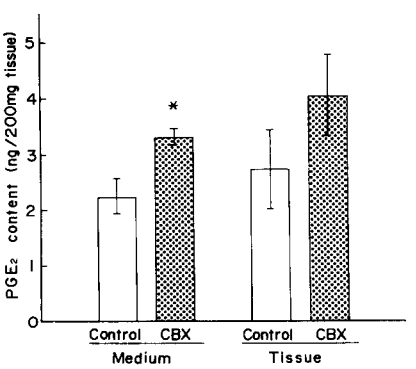

Fig. 7. Effects of CBX on PGE_2 levels in tissue and medium. A 50 μM concentration of CBX was used. Each point represents the mean ± S.E.M. of four experiments. Key: (*) $P < 0.05$, compared with each control value.

and 15-keto-PG. It has been reported by Peskar and coworkers [24, 25] that CBX inhibits 15-OH-PG-DH activity with an IC_{50} of 30 μM. Since Peskar observed little inhibition of PG synthesis by CBX, he thought that, if the combined effects of CBX on the enzymes involved in PG synthesis and inactivation were to occur *in vivo*, this would lead to elevated tissue levels of PGs. In this study, we have clearly shown an elevation of the PGE_2 levels in gastric mucosal tissue as a result of SU-88 and CBX in spite of the different mechanisms of 15-OH-PG-DH inhibition. Numerous workers have reported on the vasolidating, anti-acid secreting and cytoprotective effects of PGs in the gaster [3–8]. PGs also stimulate the gastric mucosal synthesis of mucus and its secretion [9, 10]. SU-88 produces many anti-ulcer effects such as dilation of the blood vessels in gastric mucosa, increase in the gastric local blood flow [14], stimulation of gastric mucus production [15] and elevation of the PGE_2 level in gastric mucosa, possibly due to the inhibition of the 15-OH-PG-DH activity. It is not certain whether the elevation of PGE_2 in gastric mucosa due to SU-88 involved pharmacological effects of SU-88, but considering the anti-ulcer and/or cytoprotective action of PGs it seems possible that endogeneous PGs are involved in the process of anti-ulceric and ulcer healing and may possibly be mediators in certain pharmacological actions of SU-88.

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