J. Pharm. Pharmacol. 1998, 50: 1075–1078
Received January 12, 1998
Accepted May 14, 1998

Inhibition of 15-Hydroxyprostaglandin Dehydrogenase Activity in Rabbit Gastric Antral Mucosa by Panaxynol Isolated from Oriental Medicines

YOHKO FUJIMOTO, SATORU SAKUMA, SAWAKO KOMATSU, DAISUKE SATO, HIROKO NISHIDA, YONG-QING XIAO*, KIMIYE BABA* AND TADASHI FUJITA

*Department of Hygienic Chemistry and *Department of Pharmacognosy, Osaka University of Pharmaceutical Sciences, Nasahara, Takatsuki, Osaka 569-1094, Japan*

Abstract

Panaxynol is a polyacetylene compound with anti-inflammatory and anti-platelet-aggregatory effects isolated from commonly used oriental medicines. The effects of panaxynol on the activity of prostaglandin-synthesizing and catabolizing enzymes in the rabbit gastric antral mucosa have been examined.

At concentrations ranging from 25 to 200 μM panaxynol had no effect on the synthesis of prostaglandins E_2, $F_{2\alpha}$ and D_2 from exogenous arachidonic acid in the microsomal fraction of the gastric mucosa whereas at 25–200 μM it dose-dependently inhibited the activity of 15-hydroxyprostaglandin dehydrogenase (PGDH), which catalyses the initial step of prostaglandin catabolism, in the cytosolic fraction. The concentration required for 50% inhibition (IC50) was approximately 25 μM. Inhibition of PGDH by panaxynol was non-competitive with regard to NAD^+ and prostaglandin E_2.

These results suggest that panaxynol has the potential to inhibit PGDH activity in gastric mucosa, possibly as a result of pharmacological activity.

Panaxynol (9Z-heptadeca-1,9-dien-4,6-diyn-3-ol;) is a polyacetylene compound isolated from Ginseng radix, Fang-Feng and Panax ginseng (Baba et al 1987; Teng et al 1989), commonly used oriental medicines. The anti-inflammatory and anti-platelet-aggregatory action of panaxynol have been reported (Baba et al 1987; Teng et al 1989). Recently, Alanko et al (1994) have reported that panaxynol inhibits the activity of some lipoxy-genases (5-, 12- and 15-lipoxygenases) without affecting cyclooxygenase activity.

There is much evidence to indicate that endogenous prostaglandins are important in gastric mucosal cytoprotection and other gastric functions (Main & Whittle 1973; Miller & Jacobson 1979; Robert 1979). We have previously reported that the gastric mucosa of the rabbit has a relatively high capacity for synthesis of prostaglandins (Fujimoto et al 1990). In this work we further show that the capacity of the antral region of the mucosa to synthesize prostaglandins is several times greater than that of the corporal region. Large amounts of enzymes involved in prostaglandin synthesis occur in the microsomal fraction of cells. Because prostaglandin-catabolizing enzymes, 15-hydroxyprostaglandin dehydrogenase (PGDH) and prostaglandin $\Delta 13$ reductase have been found in a 105 000 g supernatant (cytosolic fraction) prepared from rabbit stomach (Hansen 1976; Moore & Hoult 1978), prostaglandin release from the gastric mucosa depends not only on synthesis supported by enzymes catalysing prostaglandin formation, but also on enzymes associated with prostaglandin catabolism. It has also been shown that in the rabbit the antral mucosa contains large amounts of prostaglandin-catabolizing enzymes, PGDH and prostaglandin $\Delta 13$ reductase (Spenney 1979a,b). Because there have been few reports of studies of the action of panaxynol on prostaglandin synthesis and catabolism in gastric mucosa we were prompted to examine the effect of panaxynol on the activity of prostaglandin synthesizing and catabolizing enzymes in rabbit gastric antral mucosa.

Correspondence: Y. Fujimoto, Department of Hygienic Chemistry, Osaka University of Pharmaceutical Sciences, Nasahara, Takatsuki, Osaka 569-1094, Japan.

Materials and Methods

Materials

Panaxynol was isolated and purified from Saposhnikoviae radix as described elsewhere (Baba et al 1987) and dissolved in ethanol. The final

concentration of ethanol in the reaction mixture was 0·05% (v/v) in all experiments. Ethanol at this concentration had no effect on the activity of prostaglandin synthesizing and catabolizing enzymes in antral mucosal microsomes or the cytosol. Prostaglandins E_2, $F_{2\alpha}$ and D_2 (PGE_2, $PGF_{2\alpha}$ and PGD_2, respectively), the sodium salt of arachidonic acid, and indomethacin were obtained from Sigma (St Louis, MO) and 15-keto PGE_2 and 13,14-dihydro-15-keto PGE_2 were obtained from Cayman (Michigan, MI). Reduced glutathione, hydroquinone and NAD^+ were purchased from Wako (Osaka, Japan). 9-Anthryldiazomethane was obtained from Funakoshi (Tokyo, Japan). Other reagents were of analytical grade.

Preparation of microsomal and cytosolic fractions from gastric mucosa

Male rabbits, 2–2·5 kg, were anaesthetized (sodium pentobarbital, 30 mg kg^{-1}) and the whole stomachs were removed, rinsed with distilled water and rapidly chilled in ice-cold 0·9% NaCl solution (saline). The antral mucosa was obtained by gently stripping it from the underlying muscular layer and was then homogenized in 2 vols 0·1 M Tris-HCl buffer (pH 7·5). The microsomal and cytosolic fractions were prepared by the method of Tai et al (1976). The antral mucosal homogenate was centrifuged at 700*g* for 10 min in a Hitachi model 20PR refrigerated centrifuge. The supernatant was then centrifuged at 8500*g* for 10 min in the same centrifuge. The supernatant was then further centrifuged at 105 000*g* for 60 min in a Beckman model TL-100 ultracentrifuge. The supernatant thus prepared was used as the cytosolic fraction; the pellet, resuspended in 0·1 M Tris-HCl buffer (pH 7·5), was used as the microsomal fraction. Protein in the microsomal or cytosolic fractions was determined by the method of Lowry et al (1951) using bovine serum albumin as standard.

Assay of prostaglandin synthesizing enzyme activity

Antral mucosal microsomes (0·5 mg protein) were incubated with arachidonic acid (20 μM) for 15 min at 37°C in Tris-HCl buffer (0·1 M, pH 8·0; 1 mL) containing hydroquinone (0·1 mM) and glutathione (1 mM). The reaction was terminated by the addition of 6 vols petroleum ether and the aqueous phase was then acidified (approx. pH 3) with 0·5 M HCl and extracted with 6 vols ethyl acetate. PGE_2, $PGF_{2\alpha}$ and PGD_2 in the extracted lipid were determined simultaneously by the high-performance liquid chromatographic (HPLC) method described in our previous paper (Fujimoto et al 1990). Briefly, prostaglandins were measured after esterification with anthryldiazomethane. Because anthryldiazomethane contains many impurities which interfere with the HPLC determination, purification of the prostaglandin esters was attempted by the use of a normal-phase silica cartridge (Sep-Pak, Waters Associates). After preparation of the cartridge by rinsing with methanol (10 mL) then toluene–chloroform (1 : 1, v/v; 20 mL), the sample was passed through the cartridge which was washed with toluene–chloroform (1 : 1, v/v; 10 mL). The prostaglandin anthryldiazomethane derivatives were then eluted quantitatively with acetonitrile–methanol (4 : 1, v/v; 10 mL). For quantification of the derivatives peak heights were measured relative to those of standard derivatives prepared from authentic prostaglandins.

Assay for prostaglandin-catabolizing enzyme activity

Assay of prostaglandin-catabolizing enzyme activity was performed in accordance with our previously reported method (Sakuma et al 1996). Antral mucosal cytosol (0·3 mg protein) was incubated with PGE_2 (28·4 μM) for 15 min at 37°C in Tris-HCl buffer (pH 7·5, 0·05 M; 1 mL) containing 2 mM NAD^+. After incubation, 15-keto PGE_2 and 13,14-dihydro-15-keto PGE_2 in the reaction mixture were extracted and simultaneously determined by HPLC as described above.

Statistical analysis

Results are presented as means ± s.e.m. Statistical significance was determined by use of Student's *t*-test.

Results

Figure 1a shows the effect of panaxynol on prostaglandin synthesis in rabbit gastric antral mucosal microsomes. Incubation was performed in Tris-HCl buffer (0·1 M) supplemented with a mixture of compounds (glutathione and hydroquinone) previously shown to stimulate the conversion of arachidonic acid into prostaglandins by the microsomes of a variety of tissues (Takeguchi et al 1971; Tai et al 1976). In the control experiment, incubation with arachidonic acid (20 μM) in the absence of panaxynol, the major prostaglandin produced was PGE_2 (66·7%) with two other prostaglandins ($PGF_{2\alpha}$, 21·5%; PGD_2, 11·8%) also present. Panaxynol had no effect on the total prostaglandin formation (the sum of PGE_2, $PGF_{2\alpha}$ and PGD_2), reflecting cyclooxygenase activity at concentrations up to 200 μM. No significant alterations were observed in the synthesis of all three prostaglandins (PGE_2, $PGF_{2\alpha}$ or PGD_2).

Figure 1b shows the effect of panaxynol on prostaglandin catabolism in rabbit gastric antral mucosal cytosol. Incubation was performed in Tris-HCl buffer (0·05 M) supplemented with NAD^+ as a cofactor of PGDH. In the control experiment, incubation with PGE_2 (28·4 μM) in the absence of panaxynol, the major prostaglandin catabolite was 15-keto PGE_2 (81·9%) with 13,14-dihydro-15-keto PGE_2 (18·1%) also present. Panaxynol dose-dependently reduced the amount of 15-keto and 13,14-dihydro-15-keto PGE_2 formed from exogenous PGE_2, reflecting PGDH activity at concentrations ranging from 25 to 200 μM (57–91% inhibition). The concentration required for 50% inhibition (IC50) was approximately 25 μM. The inhibition was reflected in the formation of the two catabolites.

Indomethacin is a representative cyclooxygenase inhibitor known to inhibit PGDH from the rabbit lung and dog spleen (Flower 1974) and so the effects of panaxynol on the activity of prostaglandin-synthesizing and catabolizing enzymes in the gastric mucosa were compared with the effects of indomethacin (Figures 1c, d). Indomethacin (25–200 μM) dose-dependently reduced total prostaglandin formation. The IC50 value for cyclooxygenase was approximately 25 μM. The inhibition by indomethacin was reflected in the synthesis of all three prostaglandins (PGE_2, $PGF_{2\alpha}$ and PGD_2); indomethacin at concentrations of 100 μM or more reduced the formation of 15-keto PGE_2, 13,14-dihydro-15-keto PGE_2 and their sum (15-keto PGE_2 plus 13,14-dihydro-15-keto PGE_2). The IC50 value for PGDH was 150 μM. Thus, indomethacin inhibited PGDH with a higher IC50 value than panaxynol. It should be noted that indomethacin inhibited cyclooxygenase more effectively than PGDH.

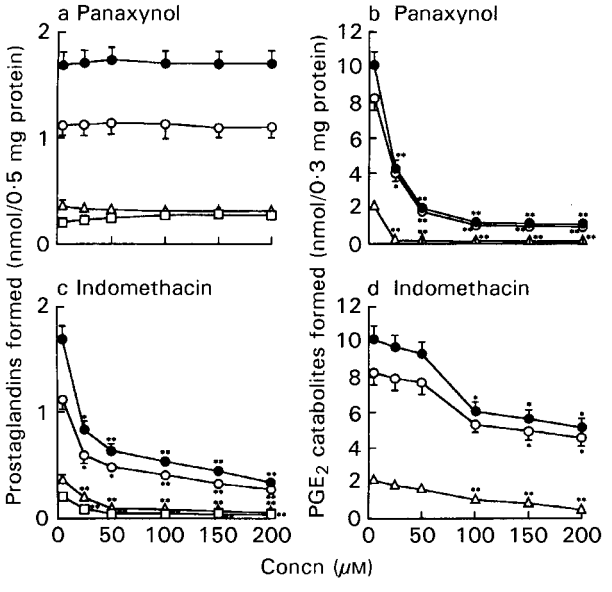

Figure 1. The effects of panaxynol and indomethacin on prostaglandin synthesis (a, c) and catabolism (b, d) in rabbit gastric antral mucosa. In a and c the microsomal fraction (0·5 mg protein) was incubated with arachidonic acid (20 μM) for 15 min at 37°C in Tris-HCl buffer (pH 8·0, 0·1 M; 1·0 mL) containing reduced glutathione (1·0 mM) and hydroquinone (0·1 mM) in the absence or presence of panaxynol or indomethacin. PGE_2, ○; $PGF_{2\alpha}$, △; PGD_2, □; the sum of PGE_2, $PGF_{2\alpha}$ and PGD_2, ●. In b and d the cytosolic fraction (0·3 mg protein) was incubated with PGE_2 (28·4 μM) for 15 min at 37°C in Tris-HCl buffer (pH 7·5, 0·05 M; 1·0 mL) containing NAD^+ (2·0 mM) in the absence or presence of panaxynol or indomethacin. 15-Keto PGE_2, ○; 13,14-dihydro-15-keto PGE_2, △; the sum of 15-keto PGE_2 and 13,14-dihydro-15-keto PGE_2, ●. Each point is the mean of results from four experiments; vertical lines show s.e.m. * $P < 0.05$, ** $P < 0.01$, significantly different from the corresponding result in the absence of panaxynol or indomethacin.

In an attempt to discover the mode of action of panaxynol as an inhibitor of PGDH, we also investigated the effect of panaxynol on PGDH activity by kinetic analysis. PGDH activity, measured by determining the sum of 15-keto PGE_2 and 13,14-dihydro-15-keto PGE_2, was linearly dependent on protein concentration (0·1–0·6 mg) and time up to 30 min (data not shown). Figure 2 shows the double-reciprocal plots of the effects of panaxynol. When NAD^+ was the variable substrate, panaxynol considerably reduced the maximum velocity of the reaction (V_{max}) but did not affect the Michaelis constant (K_m), i.e. inhibition by panaxynol seemed to be non-competitive. A similar inhibition pattern was also obtained for PGE_2.

Discussion

Because the gastric mucosa can synthesize and inactivate prostaglandins, prostaglandins that emerge from the gastric mucosa are the net result of these two opposite properties. The conversion of arachidonic acid to PGE_2, $PGF_{2\alpha}$ or PGD_2 can be separated essentially into two components. Firstly, cyclooxygenase catalyses the oxygenation of arachidonic acid to PGG_2 and the subsequent reduction of PGG_2 to PGH_2. Secondly, an endoperoxide E_2 isomerase catalyses rearrangement of PGH_2 into PGE_2, an endoperoxide $F_{2\alpha}$ reductase catalyses reduction of PGH_2 into $PGF_{2\alpha}$, or an endoperoxide D_2 isomerase catalyses rearrangement of PGH_2 into PGD_2. The first step of the catabolism of prostaglandins is catalysed by PGDH which converts prostaglandins to 15-keto prostaglandins which are, in turn, converted to 13,14-dihydro-15-keto prostaglandins by prostaglandin Δ13 reductase (Hansen 1976; Moore & Hoult 1978). PGDH has been considered as the key controlling enzyme in the catabolic pathway of prostaglandins (Samuelsson et al 1971). We examined the effects of panaxynol on the activity of prostaglandin-synthesizing and catabolizing enzymes in rabbit gastric antral mucosa,

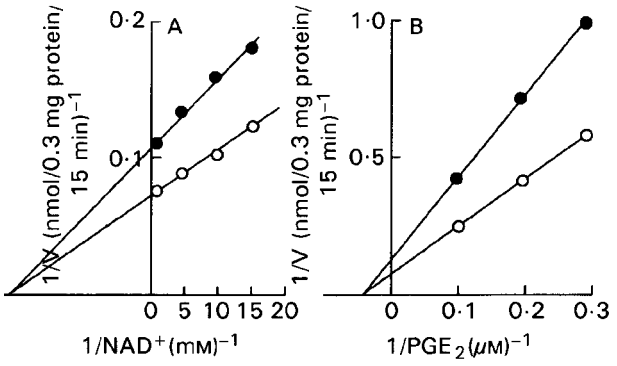

Figure 2. Double-reciprocal plots of the effects of panaxynol on 15-hydroxyprostaglandin dehydrogenase activity in rabbit gastric antral mucosa. A. PGE_2 concentration was fixed at 28·4 μM. B. NAD^+ concentration was fixed at 2 mM. Control, ○; 25 μM panaxynol, ●. Each point is the mean of duplicate experiments.

and compared the results with those for indomethacin.

As shown in Figure 1, indomethacin was a relatively poor inhibitor of PGDH when compared with its effectiveness as an inhibitor of the cyclooxygenase in the gastric mucosa. A similar tendency has been described for enzyme preparations from different tissues (Flower & Vane 1974; Hansen 1976). The current study showed that panaxynol was a more powerful inhibitor of PGDH activity in the gastric mucosa than was indomethacin, without affecting cyclooxygenase activity. The kinetic data in Figure 2 indicate that panaxynol is a non-competitive inhibitor of PGDH with regard to NAD^+ and PGE_2. This suggests that panaxynol might not affect the binding of the substrates to the enzyme. Previous studies on bovine lung PGDH (Hansen 1974) and placental PGDH from man (Jarabak 1988) have shown that indomethacin is also a non-competitive inhibitor for PGDH with regard to NAD^+ and PGE_1.

Exogenous administration of certain types of prostaglandin, such as PGE_2 and PGI_2, effectively prevent the ulceration induced by various ulcerogens (Miller & Jacobson 1979; Robert 1979; Robert et al 1979), suggesting pharmacological action of panaxynol related to its ability to modulate prostaglandin catabolism. Although the mechanisms of modulation remain to be investigated, we have obtained the first direct evidence that panaxynol, a polyacetylene compound isolated from oriental medicines, has the potential to inhibit PGDH activity in gastric mucosa.

References

Alanko, J., Kurahashi, Y., Yoshimoto, T., Yamamoto, S., Baba, K. (1994) Panaxynol, a polyacetylene compound isolated from oriental medicines, inhibits mammalian lipoxygenases. Biochem. Pharmacol. 48: 1979–1981

Baba, K., Tabata, Y., Kozawa, M., Kimura, Y., Arichi, S. (1987) Studies on chinese traditional medicine "Fang-Feng" (I) Structures and physiological activities of polyacetylene compounds from Saposhnikoviae radix. Shoyakugaku Zasshi 41: 189–194

Flower, R. J. (1974) Drugs which inhibit prostaglandin biosynthesis. Pharmacol. Rev. 26: 33–67

Flower, R. J., Vane, J. R. (1974) Inhibition of prostaglandin biosynthesis. Biochem. Pharmacol. 23: 1439–1450

Fujimoto, Y., Nakatani, E., Minamino, H., Takahashi, M., Sakuma, S., Fujita, T. (1990) Effective use of rabbit gastric antral mucosal slices in prostaglandin synthesis and metabolism studies. Biochim. Biophys. Acta 1044: 65–69

Hansen, H. S. (1974) Inhibition by indomethacin and aspirin of 15-hydroxyprostaglandin dehydrogenase in vitro. Prostaglandins 8: 95–105

Hansen, H. S. (1976) 15-Hydroxyprostaglandin dehydrogenase. Prostaglandins 12: 647-679

Jarabak, J. (1988) Inhibition of the human placental NAD- and NADP-linked 15-hydroxy-prostaglandin dehydrogenases by nonsteroidal anti-inflammatory drugs. Prostaglandins 35: 403–411

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275

Main, I. H., Whittle, B. J. R. (1973) The effects of E and A prostaglandins on gastric mucosal blood flow and acid secretion in the rat. Br. J. Pharmacol. 49: 428–436

Miller, T. A., Jacobson, E. D. (1979) Gastrointestinal cytoprotection by prostaglandins. Gut 20: 75–87

Moore, P. K., Hoult, J. R. S. (1978) Distribution of four prostaglandin-metabolising enzymes in organs of the rabbit. Biochem. Pharmacol. 27: 1839–1842

Robert, A. (1979) Cytoprotection by prostaglandins. Gastroenterology 77: 761–767

Robert, A., Nezamis, J. E., Lancaster, C., Hanchar, A. J. (1979) Cytoprotection by prostaglandins in rats. Prevention of gastric necrosis produced by alcohol, hydrochloric acid, sodium hydroxide, hypertonic sodium chloride and thermal injury. Gastroenterology 77: 433–443

Sakuma, S., Fujimoto, Y., Miyata, Y., Ohno, M., Nishida, H., Fujita, T. (1996) Effects of Fe^{2+}, Zn^{2+}, Cu^{2+} and Se^{4+} on the synthesis and catabolism of prostaglandins in rabbit gastric antral mucosa. Prostaglandins Leukot. Essent. Fatty Acids 54: 193–197

Samuelsson, B., Granstrom, E., Green, K., Hamberg, M. (1971) Metabolism of prostaglandins. Ann. NY Acad. Sci. 180: 138–163

Spenney, J. G. (1979a) Prostaglandin catabolism in fundic and antral gastric mucosa: differential content of 15-hydroxyprostaglandin dehydrogenase (PGDH). Fed. Proc. 38: 884

Spenney, J. G. (1979b) Prostaglandin-15-hydroxy-dehydrogenase and Δ13 reductase: differential content in rabbit fundic and antral mucosa and muscle. Gastroenterology 76: 1254

Tai, H. H., Tai, C. L., Hollander, C. S. (1976) Biosynthesis of prostaglandins in rabbit kidney medulla. Properties of prostaglandin synthase. Biochem. J. 154: 257–264

Takeguchi, C., Kohno, E., Sih, C. J. (1971) Mechanism of prostaglandin biosynthesis. I. Characterization and assay of bovine prostaglandin synthetase. Biochemistry 10: 2372–2376

Teng, C. M., Kuo, S. C., Ko, F. N., Lee, J. C., Lee, L. G., Chen, S. C., Huang, T. F. (1989) Antiplatelet actions of panaxynol and ginsenosides isolated from ginseng. Biochim. Biophys. Acta 990: 315–320