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## Short Communication

# PANAXYNOL, A POLYACETYLENE COMPOUND ISOLATED FROM ORIENTAL MEDICINES, INHIBITS MAMMALIAN LIPOXYGENASES

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**Abstract**—Panaxynol is a polyacetylene compound isolated from commonly used oriental medicines, and its effects on various cyclooxygenases and lipoxygenases were investigated. The compound had only a marginal effect on cyclooxygenase activities ($IC_{50}$ values $\geqslant 100\ \mu M$), but inhibited lipoxygenases; 5-lipoxygenase ($IC_{50}$, $2\ \mu M$), two isoforms of 12-lipoxygenase (leukocyte-type, $1\ \mu M$; platelet-type, $67\ \mu M$) and 15-lipoxygenase ($4\ \mu M$). Thus, panaxynol inhibited leukocyte-type 12-lipoxygenase much more effectively than platelet-type 12-lipoxygenase. Falcarindiol, an analogue of panaxynol, inhibited these lipoxygenases with higher $IC_{50}$ values than panaxynol. These compounds could provide a clue to develop a selective inhibitor of one isoform of 12-lipoxygenase.

*Key words:* arachidonic acid; cyclooxygenase; falcarindiol; platelets; leukocytes

Panaxynol [(9*Z*)-heptadeca-1,9-dien-4,6-diyn-3-ol] was isolated from Ginseng radix, "Fang-Feng" and Panax ginseng [1, 2] and falcarindiol [(9*Z*)-heptadeca-1,9-dien-4,6-diyn-3,8-diol] from "Fang-Feng" [1] (Fig. 1). They are commonly used oriental medicines. Anti-inflammatory and anti-platelet-aggregatory actions of panaxynol were previously reported [1, 2]. In view of their 1,9-dien-4,6-diyn structure as an analogue of the polyen structure of arachidonic acid, we investigated their effects on various mammalian cyclooxygenases and lipoxygenases. Cyclooxygenase incorporates two molecules of oxygen into arachidonic acid, and initiates the biosynthesis of prostaglandins and thromboxanes [3]. Lipoxygenase incorporates only one molecule of oxygen at particular positions of unsaturated fatty acid [4, 5]. Biologically potent leukotrienes are synthesized via the 5-lipoxygenase pathway of arachidonic acid [6]. 15-Lipoxygenase was found in leukocytes [7] and reticulocytes [8]. There are two types of 12-lipoxygenase (platelet-type and leukocyte-type), which are distinguishable in terms of substrate specificity, immunogenicity and amino acid sequence [4, 5].

### *Materials and Methods*

[1-$^{14}$C]Arachidonic acid (2.04 GBq/mmol) was purchased from Amersham International (Amersham, U.K.), and unlabeled arachidonic acid from Nu-Chek Prep (Elysian, MN, U.S.A.). Panaxynol and falcarindiol were isolated and purified from Saposhnikoviae radix as described previously [1]. Sheep cyclooxygenase purified from seminal vesicle by immunoaffinity chromatography was supplied by K. Yamamoto of this laboratory. A suspension of platelet microsomes was used as human cyclooxygenase. Porcine 5-lipoxygenase [9] and 12-lipoxygenase [10] were purified from leukocytes by immunoaffinity chromatography as described. Recombinant 12-lipoxygenase of human platelets was prepared by M. Nakamura and K. Kishimoto of this laboratory. 15-Lipoxygenase was a lysate from rabbit reticulocytes.

The standard assay mixtures ($200\ \mu L$) were as follows: for cyclooxygenase 0.1 M Tris–HCl buffer (pH 8.0), $2\ \mu M$ hematin and 5 mM tryptophan; for 12- and 15-lipoxygenases 0.05 M Tris–HCl buffer (pH 7.5); and for 5-lipoxygenase 0.05 M potassium phosphate buffer (pH 7.4), 2 mM $CaCl_2$ and 2 mM ATP. The enzyme amount was adjusted so that approx. 50% of arachidonic acid could be oxygenated. The reaction was started by the addition of [1-$^{14}$C]arachidonic acid (100,000 cpm/5 nmol in $5\ \mu L$ of ethanol). Cyclooxygenase reaction was performed at 24° for 1 min, and lipoxygenase reactions at 30° for 5 min. Incubation was stopped by adding $300\ \mu L$ ice-cold diethyl ether/methanol/0.2 M citrate (30:4:1, by vol.). Thin layer chromatography was performed as described previously with a solvent system of diethyl ether/petroleum ether/acetic acid (85:15:0.1 by vol.) [9]. Panaxynol and falcarindiol were added in $4\ \mu L$ of dimethyl sulfoxide.

### *Results and Discussion*

Panaxynol had essentially no effect on ovine and human cyclooxygenases up to $30\ \mu M$ concentration except for a slight activation of the enzyme activity at lower concentrations (Fig. 2). $IC_{50}$ values were higher than $100\ \mu M$. In contrast, panaxynol inhibited various lipoxygenases (Fig. 2). $IC_{50}$ values for 5-lipoxygenase, 12-lipoxygenases of leukocyte-type and platelet-type and 15-lipoxygenase were 2, 1, 67 and $4\ \mu M$, respectively. The most potent inhibition was observed with leukocyte-type 12-lipoxygenase, and the compound was slightly less effective on 5- and 15-lipoxygenases. It should be noted that platelet-type 12-lipoxygenase required approx. 70 times higher concentration of the inhibitor than leukocyte-type 12-lipoxygenase.

As described above, cyclooxygenases and lipoxygenases were distinguished in terms of the inhibition by panaxynol. However, panaxynol is not a selective inhibitor for a particular lipoxygenase, inhibiting as it does 5-, 12- and 15-lipoxygenases. Two 12-lipoxygenases of porcine leukocytes and human platelets were inhibited by panaxynol with $IC_{50}$

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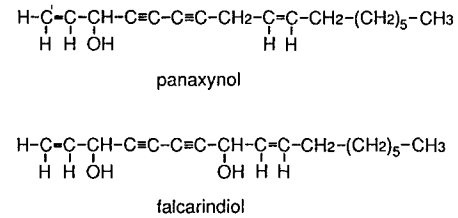


Fig. 1. Structures of panaxynol and falcarindiol.

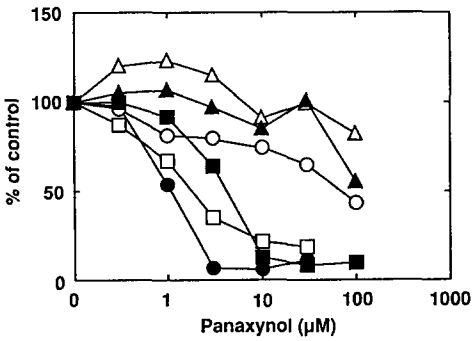


Fig. 2. Effects of panaxynol on various preparations of cyclooxygenase and lipoxygenase. The following amounts of enzymes were assayed with arachidonic acid under the standard conditions; cyclooxygenase of ovine seminal vesicle (open triangles, 1.0 μg protein), cyclooxygenase of human platelets (closed triangles, 148 μg protein), 5-lipoxygenase of porcine leukocytes (open squares, 8.0 μg protein), 12-lipoxygenase of porcine leukocytes (closed circles, 2.0 μg protein), recombinant 12-lipoxygenase of human platelets (open circles, 0.7 μg protein) and 15-lipoxygenase of rabbit reticulocytes (closed squares, 16.1 μg protein). Mean of four to five experiments.

values different by almost two orders of magnitude. This finding would provide a clue to develop a selective inhibitor to distinguish the two types of 12-lipoxygenase. Such a selective 12-lipoxygenase inhibitor would help to elucidate still unknown physiological roles of 12-lipoxygenases.

Falcarindiol is an analogue of panaxynol with an additional hydroxyl group at 8 position (Fig. 1). The compound was a less effective inhibitor of the lipoxygenases tested above. $IC_{50}$ values for 5-lipoxygenase, 12-lipoxygenases of leukocyte-type and platelet-type and 15-lipoxygenase were 7, 48, >100 and 18 μM, respectively. Falcarindiol had essentially no effect on either ovine or human cyclooxygenases up to 30 μM concentration.

Eicosa-5,8,11,14-tetraynoic acid was earlier shown to inactivate cyclooxygenase [11] and lipoxygenase [11, 12] by its covalent binding to the enzyme proteins. Given its 1,9-dien-4,6-diyn structure, panaxynol was subjected to a kinetic study with porcine leukocyte 12-lipoxygenase which was inhibited most potently by the compound. Since the enzyme is known as a suicide enzyme and the reaction time course is almost linear for only 1 min or so, the enzyme reaction was performed for 1 min. Panaxynol was shown to be a competitive inhibitor as examined by Lineweaver–Burk plots. Such a competitive nature suggests that panaxynol is bound to the active site of the enzyme as a substrate analogue. However, it is possible that the inhibitor is covalently bound to the enzyme protein by its diyn portion. Preincubation of porcine leukocyte 12-lipoxygenase with panaxynol was carried out on ice because of the enzyme lability. $IC_{50}$ values after 30 and 60 min preincubations were 0.7 and 0.5 μM, respectively, as compared with 1 μM after 1 min preincubation. The enzyme activity decreased by 60 and 76% after incubation for 30 and 60 min. It is not certain whether such a slow inactivation may be attributed to a slow covalent binding of the inhibitor to the enzyme protein. First, panaxynol interacts with the active site of 12-lipoxygenase competing with arachidonic acid, and then may be gradually covalently bound to the enzyme protein to be an irreversible inhibitor.

As examined previously with whole platelet cells, the production of thromboxane $B_2$ [1, 2] and 12-hydroxy-5,8,10-heptadecatrienoic acid [2] was inhibited by panaxynol at 0.4–0.8 mM [1] and 0.08–0.8 mM [2]. At such high concentrations cyclooxygenase activity may be inhibited by panaxynol, resulting in the reduced production of thromboxane $B_2$. The lipoxygenase inhibition observed in the present work required much lower concentrations of panaxynol.

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