Structure-function relationships of non-cyclic dioxygenase products from polyunsaturated fatty acids: Poxytrins as a class of bioactive derivatives.

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Abstract

More and more attention is paid to omega-3 fatty acids because of their potential activities in preventing cardiovascular events. In this brief review, we focus on the lipoxygenase end-metabolites of two relevant nutrients belonging to the omega-3 family fatty acids: alpha-linolenic and docosahexaenoic acids, the latter being a prominent component of brain lipids. Dihydroxylated derivatives are described as well as their inhibitory effects on platelet aggregation and cyclooxygenase activities. We point out that only the dihydroxylated products with the *trans,cis,trans*/*E,Z,E* conjugated triene geometry exhibit those inhibitory activities. These properties being found with other polyunsaturated fatty acid oxygenated products sharing the same *E,Z,E* molecular motif, they have been collectively named poxytrins. From alpha-linolenic and docosahexaenoic acids, poxytrins are linotrins and protectin DX, respectively.

Introduction

Most polyunsaturated fatty acids (PUFA) of the omega-6 and -3 families are considered having essential functions, and they derive from the indispensable precursors linoleic (18:2ω6) and alpha-linolenic (18:3ω3) acids in humans. They can be metabolized into longer and highly unsaturated fatty acid derivatives by a series of desaturases and elongases [1]. All the PUFA have at least two methylene-interrupted double-bonds, which is the required molecular motif, called 1,4-*cis,cis*/*Z,Z*-pentadiene, to be oxygenated by lipoxygenases or by non-enzyme peroxidation [2].
Lipoxygenases have been first described in plants, and 18:2ω6 is a well-known substrate of soybean lipoxygenase to produce 13-hydroperoxy-octadecadienoic acid (13-HpODE), and considered to be a better substrate than arachidonic acid (20:4ω6) [3]. Then, animal lipoxygenases have been reported to mainly oxygenate 20:4ω6 at carbon 5, 12 and 15 giving 5-, 12- and 15-hydroperoxy-eicosa-tetraenoic acids (HpETE), respectively, that are reduced into the corresponding alcohols (HETE) by glutathione peroxidase (GPx), usually the cytosolic isoform (GPx-1) [4]. The stereo-chemistry of those products is usually *S* when lipoxygenases are the catalysts, whereas racemic (*R/S*) products are issued from non-enzyme peroxidation [5]. In some cases, the product of one lipoxygenase may be further oxygenated by a second one, e.g. 5(*S*)-HETE may be further converted into 5(*S*),12(*S*)-diHETE by 12-lipoxygenase or *vice versa*. In addition, 15-lipoxygenase may act twice, first producing 15(*S*)-HETE, and second at position 8, making a double lipoxygenase product: 8(*S*),15(*S*)-diHETE [6]. In terms of double-bond geometry, the mono-oxygenated products are *E,Z* conjugated dienes with the oxygenation closest to the *E* double-bond, and the double oxygenated products are *E,Z,E* conjugated trienes with the oxygenation adjacent to the *E* double-bonds. Thus, the double lipoxygenase end-product 5(*S*),12(*S*)-*E,Z,E*-diHETE, sometimes called leukotriene Bx, is a stereo-chemical and geometric isomer of leukotriene B_4 (5(*S*),12(*R*)-*Z,E,E*-diHETE) [7,8].

Lipoxygenation of docosahexaenoic acid into protectins

Docosahexaenoic acid (DHA) is a major PUFA of the omega-3 family. It is the main PUFA in structural lipids in the brain and retina where it plays important roles in brain development, learning ability and visual acuity [9]. As a main nutrient from marine glycerolipids, DHA is known as well for its protective effects against athero-thrombosis and inflammation [10]. It is not a substrate of cyclooxygenases but inhibits them by competing with arachidonic acid [11]. In contrast, it is a fairly good substrate of lipoxygenases leading to the end-products hydroxyl-docosa-hexaenoic acids (HDoHE): 4- and 7-HDoHE through 5-lipoxygenase [12, 13], 11- and 14-HDoHE through 12-/ω9-lipoxygenase [14] and 17-HDoHE through 15-/ω6-lipoxygenase [15]. In addition to those mono-oxygenations, DHA may undergo a double oxygenation by 15-/ω6-lipoxygenase. The end-product is 10(*S*),17(*S*)-dihydroxy-4*Z*,7*Z*,11*E*,13*Z*,15*E*,19*Z*-docosahexaenoic acid [16]. This product has been named protectin DX (PDX) to differentiate it from protectin D1 (PD1), also called neuroprotectin D1 (NPD1) because of its occurrence in the brain where it exert neuroprotective activities [17]. PD1 (10(*R*),17(*S*)-dihydroxy-4*Z*,7*Z*,11*E*,13*E*,15*Z*,19*Z*-docosahexaenoic acid) differs from PDX both by the stereochemistry of carbon 10 (*R* instead of *S*) and the conjugated triene geometry (*E*,*E*,*Z* instead of *E*,*Z*,*E*) [18].

Like all the other *E*,*Z*,*E* conjugated trienes (named poxytrins®, Figure 1) tested, including the other double lipoxygenation end-products 8*S*,15*S*-diHETE from 20:4ω6, 10(*S*),17(*S*)-di-OH-22:3 from 22:3ω6 and 7(*S*),14(*S*)-di-OH-22:6, the latter being a geometric and stereo-isomer of maresin 1[19], PDX inhibits human blood platelet aggregation induced by both collagen and thromboxane. As collagen induces platelet aggregation *via* a cascade which includes the release of endogenous arachidonic acid from membrane phospholipids, its oxygenation into thromboxane A_2 and its response to the latter, the oxygenated metabolism of arachidonic acid was investigated. It appeared that PDX does not alter the release of arachidonic acid but its oxygenation through cyclooxygenase-1, the only isoform within blood platelets [20]. However, further studies have shown that cyclooxygenase-2 is inhibited as well by PDX, with even more potency compared to cyclooxygenase-1 [21]. PDX also inhibits thromboxane A_2-induced aggregation (using the stable analog U46619 as an agonist) as it has been shown previously with inhibitors such as monohydroxy derivatives of PUFA and leukotriene Bx but not leukotriene B_4 [22]. This means that PDX inhibits both the generation of thromboxane and its aggregatory effect. Of note, the inhibitory power towards collagen and 20:4ω6 was similar, suggesting that PDX is not active against the phospholipase A_2-induced release of endogenous 20:4ω6. In all aggregation tests, it appeared that, in contrast to *E*,*Z*,*E* derivatives, the non *E*,*Z*,*E* ones tested (*E*,*E*,*Z* and *E*,*E*,*E*) are devoid of inhibitory effect [20]. This is in agreement with a previous paper reporting that PD1 (*E*,*E*,*Z* motif) is a weak inhibitor of ADP-induced platelet aggregation [23].

Very recent data, obtained in a completely different biological system, have shown that PDX, but not PD1, can improve insulin sensitivity in skeletal muscle of obese diabetic mice [24]. However, this specific effect could not be attributed solely to the *E*,*Z*,*E* motif as 8(*S*),15(*S*)-diHETE (with the *E*,*Z*,*E* geometry) was inactive [24]. This is another example of biological effect of PDX after other recent data showing that PDX (called PD1 isomer and even PD1 in most part of the text [18]) specifically inhibits the replication of the influenza virus, and improves severe influenza states [25].

Lipoxygenation of alpha-linolenic acid into linotrins

Although alpha-linolenic acid (18:3ω3, ALA) is known to exert protective effects against atherothrombogenesis [26], it is known to hardly accumulate in human blood and tissues.

Also, its conversion into long-chain omega-3 derivatives such as eicosapentaenoic acid (20:5ω3, EPA) and DHA is weak [27], preventing to consider it could mainly act through the long-chain derivatives. Finally, ALA is believed to be efficiently beta-oxidized [28], but this does not explain the majority of its metabolic fate. ALA has the structural basis for being a substrate of 15-/ω6-lipoxygenase as well as 18:2ω6, which explains part of its metabolic behavior, yet its lipoxygenation has been scarcely studied [29, 30], and the chemical characterization of the end-products in these studies was not completed. In addition, no biological activities were proposed to the oxygenated products from ALA.

The conversion of ALA by soybean lipoxygenase has been more thoroughly investigated, in conditions similar to those used to study the conversion of DHA to PDX. Substantial amount of 13(*S*)-hydroxy-9*Z*,11*E*,15*Z*-octadecatrienoic acid (13(*S*)-HOTE) was obtained, and characterized by mass spectrometry after reduction of the hydroperoxide intermediate by $NaBH_4$. In addition, four conjugated triene end-products have been found after reduction, and characterized by UV, mass spectrometry, and NMR. They are 9(*R*),16(*S*)-dihydroxy-10*E*,12*E*,14*E*-, 9(*S*),16(*S*)-dihydroxy-10*E*,12*E*,14*E*-, 9(*S*),16(*S*)-dihydroxy-10*E*,12*Z*,14*E*-, and 9(*R*),16(*S*)-dihydroxy-10*E*,12*Z*,14*E*-octadecatrienoic acids. The two latter ones have the *E*,*Z*,*E* conjugated triene feature of poxytrins (Figure 2), and were found to inhibit human blood platelet aggregation induced by collagen, whereas the two former ones are all-trans (*E*,*E*,*E*) products which are not active upon platelets [31]. The two poxytrins derived from ALA have been named "linotrins®". Interestingly, linotrin 9(*R*),16(*S*) (linotrin-1) appeared slightly more potent to inhibit platelet aggregation than linotrin 9(*S*),16(*S*) (linotrin-2) (Figure 2). They both inhibited the two cyclooxygenase isoforms COX-1 and COX-2, in their pure form, with the same highest activity for linotrin-1. In addition, the latter inhibited slightly but significantly the 5-lipoxygenase pathway tested with 20:4ω6 as a substrate in human polymorphonuclear leukocytes, which means that leukotriene B_4 production was decreased as well by this linotrin (linotrin-1). Overall, linotrins may then exert anti-atherothrombotic effects with linotrin-1 being more active [31]. The highest activity of linotrin-1 is of biological relevance because 9(*R*)-HOTE may be produced by acetylated COX-2 through aspirin treatment, whereas 9(*S*)-HOTE results from normal COX-2, both in an aborted cyclooxygenation process [32]. As we have found that 9(*R*)-HOTE and 9(*S*)-HOTE are good substrates of the recombinant human 15-/ω6-lipoxygenase [31], this makes the two linotrins interesting products, with a possible pre-eminence for linotrin-1, to explain part of the anti-atherothrombotic properties of ALA.

Conclusion and perspectives

All PUFA being potential production of R products from aspirinated COX-2 as substrates for further lipoxygenation. Figure 3 summarizes the molecular targets of poxytrins. It is worth to add that, if we consider the monohydroxylated derivatives of DHA as the most active to inhibit platelet aggregation when compared to monohydroxylated from other PUFA [34], DHA is clearly an interesting substrates of several lipoxygenases, they are candidates to be converted into poxytrins when they have at least three double-bonds with the 1*Z*,4*Z*,7*Z* octadecatriene motif. The *E*,*Z*,*E* conjugated triene in poxytrins seems to be crucial for the inhibition of COX activities, since *Z*,*E*,*E* or *E*,*E*,*Z* conjugated analogs are not active, as we have first described by comparing 5(*S*),12(*S*)-*E*,*Z*,*E*-diHETE (LTBx) with 5(*S*),12(*R*)-*Z*,*E*,*E*-diHETE (LTB_4) [22]. The relative higher inhibitory potencies of R derivatives compared with the S ones [20, 33] is promising in the frame of the possible nutrient to prevent athero-thrombogenesis.

In addition to these anti-atherothrombotic properties, poxytrins may be of interest in a totally different field. As a matter of fact, a recent paper has reported that PDX is the only oxylipin able to inhibit the influenza virus replication and combat severe influenza [25]. This is another

example of specific biological properties attributed to PDX as already mentioned above for the improvement of insulin sensitivity (24).

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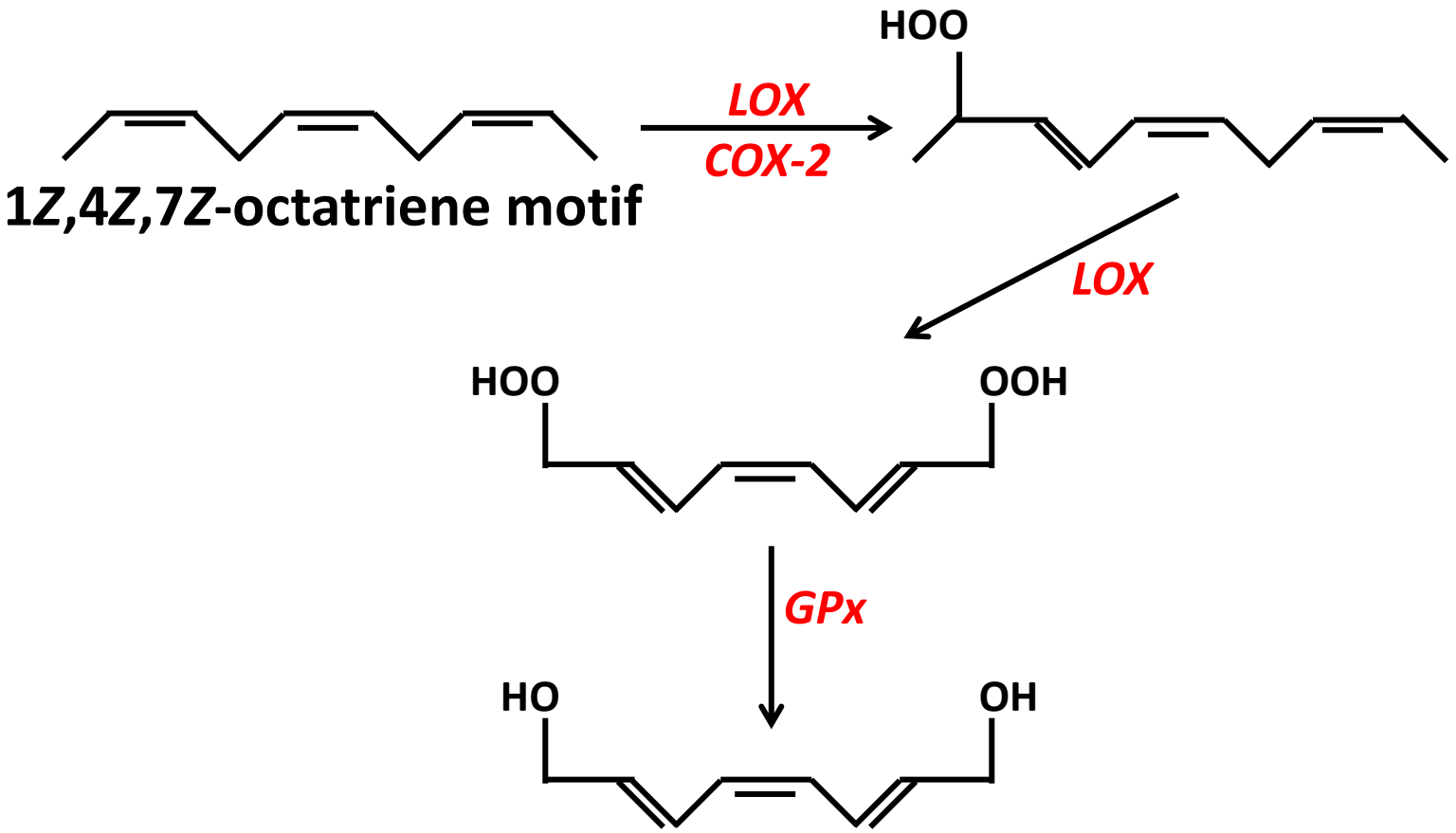
Figure legends

Figure 1: Biosynthesis of poxytrins. PUFA that possess at least three double-bonds with the 1*Z*,4*Z*,7*Z*-octatriene motif may be oxygenated twice by lipoxygenases (LOX) or first by cyclooxygenase-2 (COX-2) in an aborted cyclooxygenation process. When COX-2 is acetylated by aspirin, the stereochemistry of the oxygenated carbon is R instead of S in normal oxygenations. Then, the hydroperoxide intermediates are reduced by glutathione peroxidase (GPx) to provide the 1,8-dihydroxy-octa-2*E*,4*Z*,6*E*-triene motif of poxytrins.

Figure 2: Chemical structures of three poxytrins. They are protectin DX (10(*S*),17(*S*)-dihydroxy-4*Z*,7*Z*,11*E*,13*Z*,15*E*,19*Z*-docosahexaenoic acid) from DHA, and linotrins-1 & -2 (9(*R*),16(*S*)- & 9(*S*),16(*S*)-dihydroxy-10*E*,12*Z*,14*E*-octadecatrienoic acids, respectively) from ALA.

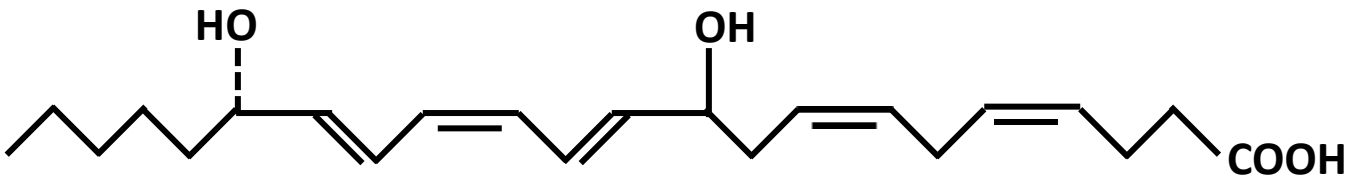
Figure 3: Effects of poxytrins on blood platelet functions. Poxytrins inhibit prostanoids synthesis (e.g. from arachidonic acid (ARA)), including that of thromboxane, through cyclooxygenase (COX) inhibition, and counteract the aggregatory effect of thromboxane A_2 (TxA_2). The biosynthesis of primary prostaglandins (i.e. PGD_2, E_2, $F_{2\alpha}$) should be inhibited as well, and their subsequent bioactivity (although this has not been tested).



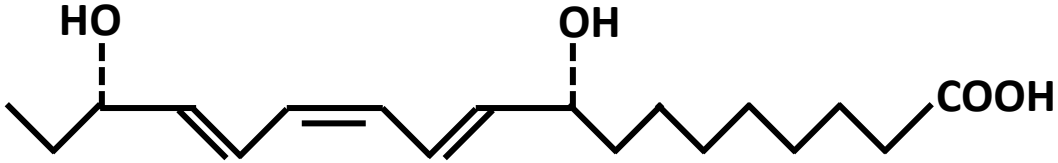
« Poxytrin® »

(1,8-dihydroxy-octa-2*E*,4*Z*,6*E*-triene motif)

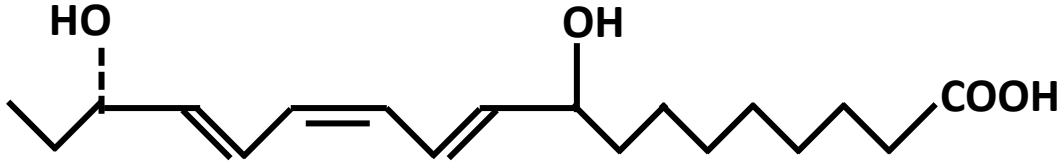
Figure 1



Protectin DX



Linotrin-1



Linotrin-2

Figure 2

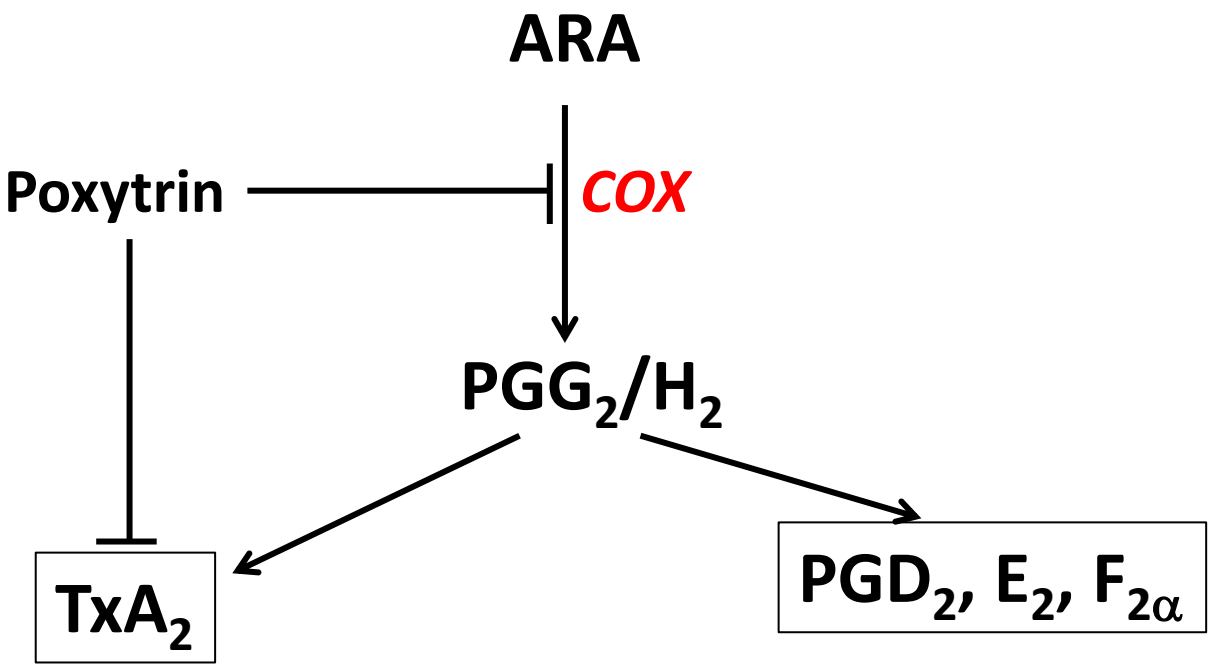


Figure 2