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Review

Anti-inflammatory and anti-virus potential of poxytrins, especially protectin DX

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ABSTRACT

Poxytrins (Pufa Oxygenated Trienes) are dihydroxy derivatives from polyunsaturated fatty acids (PUFA) with adjacent hydroxyl groups to a conjugated triene having the specific *E,Z,E* geometry. They are made by the double action of one lipoxygenase or the combined actions of two lipoxygenases, followed by reduction of the resulting hydroperoxides with glutathione peroxidase. Because of their *E,Z,E* conjugated triene, poxytrins may inhibit inflammation associated with cyclooxygenase (COX) activities, and reactive oxygen species (ROS) formation. In addition of inhibiting COX activities, at least one poxytrin, namely protectin DX (PDX) from docosahexaenoic acid (DHA), has also been reported as able to inhibit influenza virus replication by targeting its RNA metabolism.

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1. Introduction

The main natural polyunsaturated fatty acids (PUFA) have at least two methylene-interrupted *cis, cis* (*Z,Z*) double bonds, which facilitates their oxygenation by lipoxygenases. This means that one oxygen molecule is fixed to one unsaturated carbon, making the hydroperoxide R—OOH from the substrate RH. This induces the migration of the double bond associated with the oxygenated carbon toward the initially non-conjugated adjacent double bond, resulting to the conjugated *cis, trans* (*Z,R*) double bonds next to the OOH group (1*Z*,3*R*-4-OOH) (see also the first reaction in Fig. 1 as an example). This relatively unstable hydroperoxide is usually reduced into its stable hydroxylated derivative 1*Z*,3*R*-4-OH product by glutathione-dependent peroxidases.

2. Structural features

Poxytrins are double lipoxygenase products of polyunsaturated fatty acids (PUFA) [1,2]. The structural feature of poxytrins is to own three conjugated double bonds with the *trans,cis, trans* (*E,Z,E*) geometry between two secondary alcohols derived from the initial hydroperoxides produced by lipoxygenation of the initial PUFA. Because of the two lipoxygenations, the configuration of both oxygenated carbons is mainly (S).

The first example of such a structure has been given for a geometric and stereoisomer of the pro-inflammatory derivative from arachidonic acid (5,8,11,14—20:4), leukotriene B4 (LTB4), the latter

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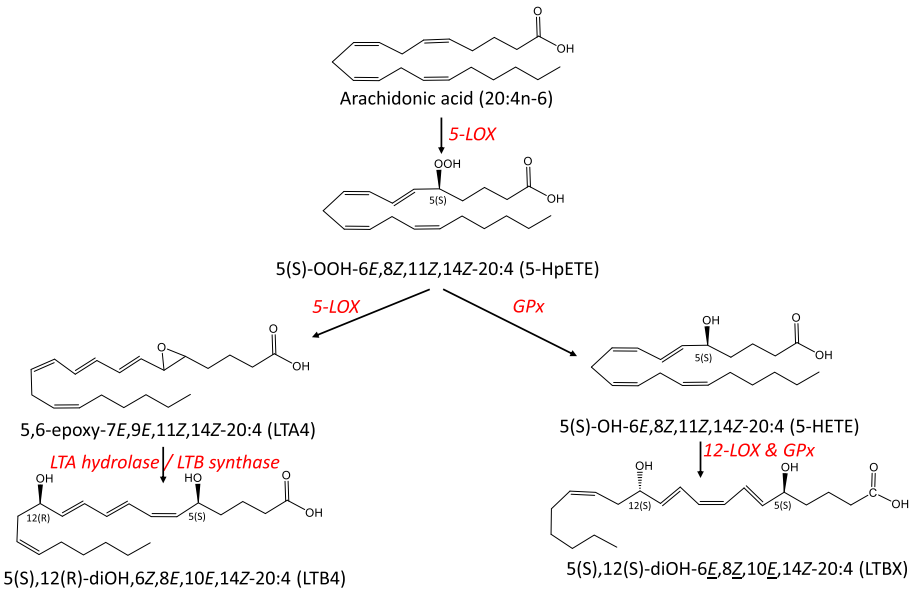

Fig. 1. Structures of compounds related to leukotriene B4 synthesis as well as to its geometric and stereoisomer LTBX. 5/12-LOX: 5/12-lipoxygenase; GPx: glutathione peroxidase.

being 5(S),12(R)-diOH,6*Z*,8*E*,10*E*,14*Z*-20:4, as described in blood leukocytes [3]. LTB4 is made by 5-lipoxygenase, which is unique to first oxygenate carbon 5 into 5(S)−OOH-6*E*,8*Z*,11*Z*,14*Z*-20:4 or 5(S)-HpETE, and second to catalyze the 5,6-epoxide formation from the hydroperoxide group, leading to 5,6-epoxy-7*E*,9*E*,11*Z*,14*Z*-20:4 (called leukotriene A4 or LTA4). Then an epoxide hydrolase, called LTA4 hydrolase or LTB4 synthase, gives rise to LTB4 [4]. In contrast, when glutathione peroxidase (GPx) reduces 5(S)-HpETE into its corresponding 5-OH derivative 5(S)-HETE, the latter product cannot be converted into the 5,6-epoxide. 5(S)-HETE then becomes available for further conversion, including by platelet 12-lipoxygenase, which leads to 5(S),12(S)-diOH-6*E*,8*Z*,10*E*,14*Z*-20:4 or 5(S),12(S)-diHETE after reduction by GPx. *Vice versa*, 12(S)-HETE from platelets may be converted by leucocyte 5-LOX into the same *E,Z,E* conjugated triene [5] (Fig. 1).

In terms of bioactivity, whereas LTB4 is a potent pro-inflammatory compound by inducing leukocytes chemotaxis, its isomer 5(S),12(S)-diHETE is much less active [6]. In addition and in contrast, when several lipoxygenase products have been tested against blood platelet aggregation, 5(S),12(S)-diHETE was found able to inhibit thromboxane-induced aggregation, like many other single lipoxygenase products, but LTB4 was totally inactive [7] (Croset & Lagarde BBRC 1982). 5(S),12(S)-diHETE was further called LTBX and belongs to the poxytrin family [2].

Other di-hydroxy derivatives of PUFA have been described as produced only by 15-lipoxygenase, which is able to oxygenate the PUFA having at least three double bonds, on two carbons, then making the conjugated triene *E,Z,E*. This has been reported for arachidonic acid, leading to 8(S),15(S)-diOH-5*Z*,9*E*,11*Z*,13*E*-20:4 [8]. Also, the same type of derivative has been described from docosahexaenoic acid (22:6n-3). A double lipoxygenation product has been first described as 10,17(S)-diOH-4*Z*,7*Z*,11*E*,13*Z*,15*E*,19*Z*-22:6 [9], and further characterized as 10(S),17(S)-diOH-4*Z*,7*Z*,11*E*,13*Z*,15*E*,19*Z*-22:6 and called protectin DX (PDX) [10]. PDX *versus* its isomer protectin D1 (PD1), which is the 10(R),17(S)-diOH-4*Z*,7*Z*,11*E*,13*E*,15*Z*,19*Z*-22:6 [11], is like LTBX *versus* LTB4, with the same (S), (S) *vs* (S), (R) stereochemical and *E,Z,E vs E,E,Z* geometric configurations (Fig. 2).

Since those reports for arachidonic and docosahexaenoic acids, the most abundant omega-3/n-3 fatty acid, alpha-linolenic acid, has been considered since it has the minimal *Z,Z,Z* structure to be doubly oxygenated. Two di-OH derivatives with the *E,Z,E* conjugated triene motif have been described from alpha-linolenic acid (18:3n-3), when dioxygenated by soybean lipoxygenase. They differ by the stereochemistry of carbon 9: 9(S),16(S)- and 9(R),16(S)-diOH-10*E*,12*Z*,14*E*-18:3 (Fig. 2). Since they share the *E,Z,E* geometry of other poxytrins, these linolenic acid derivatives have been named linotrins. Both stereoisomers share the capacity of inhibiting platelet aggregation, the 9(R),16(S) being slightly more potent than the 9(S),16(S) stereoisomer [12]. The same higher potency was found for the (R) stereoisomer of protectin DX (10(R),17(R) versus 10(S),17(S)) [1]. This observation for (R) stereoisomers poxytrins might be relevant to aspirin treatment, because 9(R)−OH−10*E*,12*Z*,15*Z*-18:3 and 17(R)−OH−4*Z*,7*Z*,10*Z*,13*Z*,15*E*,19*Z*-22:6 could be made from 18:n-3 and 22:6n-3, respectively, by cyclooxygenase-2 acetylated by aspirin, as shown for arachidonic acid [13]. Therefore, the (R) mono-hydroxy derivatives from 18:3n-3 and 22:6n-3 may be lipoxygenated into the corresponding poxytrins with the (R), (S) stereochemistry.

3. Anti-inflammatory and anti-viral potentials of poxytrins, especially PDX

The inhibition of platelet aggregation by PDX revealed that part of it was due to inhibition of cyclooxygenase-1 [1]. In a further study, it was shown that PDX inhibits both purified cyclooxygenases (COX-1 and -2), with even more activity against COX-2 [14]. This observation is particularly relevant to inflammation as COX-2, the inducible isoform of COX, is especially expressed in the inflammatory states. In addition, PDX showed a broad anti-inflammatory effect on human blood neutrophils by decreasing reactive oxygen species production and myeloperoxidase release [14]. Altogether, these inhibitory activities make protectin DX as a potential inhibitor of inflammation.

In addition to PDX, linotrins have been found able to inhibit COX-1 and COX-2 as well [12], with a slight higher activity of the (R) stereoisomer compared to the (S) one. In favor of a stronger inhibition of inflammation by the (R) stereoisomer of linotrins is the

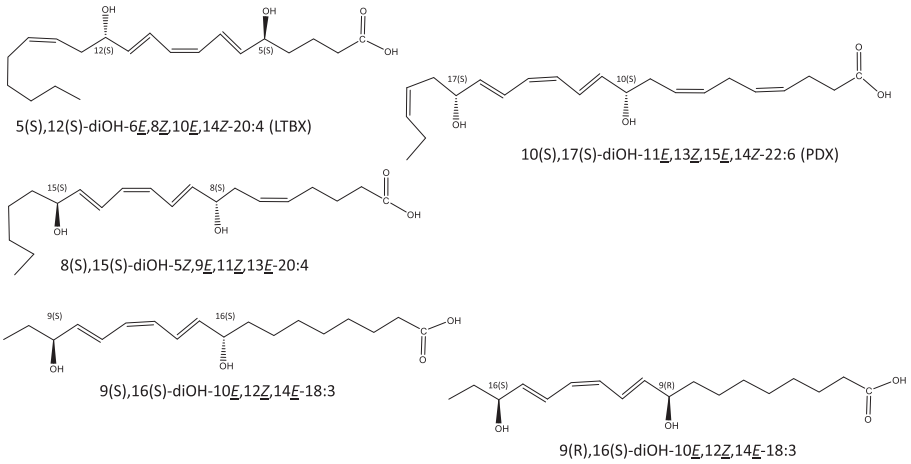

Fig. 2. Structures of double 15-lipoxygenase products from arachidonic (5,12- and 8,15-diOH-20:4), docosahexaenoic (protectin DX or PDX) and alpha-linolenic (9(S),16(S)- and 9(R),16(S)-diOH-18:3 or linotrins) acids, globally called poxytrins. They all show the characteristic configuration of the *E,Z,E,* conjugated triene between two secondary alcohols.

decreased 5-lipoxygenation of arachidonic acid, especially LTB4 formation, while the (S) stereoisomer is inactive [12] as previously observed for PDX.

Even more promising with regards to viral infections, is the fact that PDX may inhibit the replication of a RNA influenza virus [15]. It is important to state that this was really PDX, not PD1 as written in the paper (except in methods), and confirmed later by the corresponding Author in a review [16]. From the *Cell* paper [15], we may consider that the active molecular motif against the virus replication is the conjugated *E,Z,E* triene, because other oxygenated derivatives from DHA/22:6n-3 such as resolvins D1 and D2 which have a conjugated E,E,Z,E and E,Z,E,E tetraene, respectively, were completely inactive when compared with PDX. From other considerations, we could anticipate that linotrins might be good candidates because they do not have any other double bond, preventing the molecule to be further oxidized, being then more stable to be used as a "drug".

4. Targeting the brain with poxytrins

The geometric and stereoisomer of the poxytrin PDX, PD1, has also been named neuroprotectin D1, because of its neuroprotective activity [11,17]. From a lipidomic analysis of normal rat brain, it appeared that PDX can be found, like the other poxytrin 8,15-diHETE [18]. However PD1 could only be detected in inflamed rat brain in response to lipopolysaccharide (unpublished result). On the other hand, it is now well recognized that 1-lyso,2-docosahexaenoic acid-glycerophosphocholine (LysoPC-DHA) is the physiological transporter of plasma DHA to the brain [19,20]. Also, it is known that DHA moves from the *sn-2* position to the *sn-1* position of LysoPC-DHA, with the latter position isomer representing three quarters of the total [21]. We then prevented such an isomerization by acetylating the sn-1 position of LysoPC-DHA [22]. The resulting structured phospholipid has been named AceDoPC, and further studies have shown that AceDoPC remains a preferred vehicle of DHA to the brain, like LysoPC-DHA [23]. Considering the bioactive potential of PDX, we have made and characterized a poxytrin-derived from AceDoPC, named AceDoxyPC, in which DHA is replaced by PDX [24]. Investigations remain to evaluate the brain uptake of PDX within AceDoxyPC, but the bioactivity potential of PDX, anti-inflammatory effect and antiviral potential, makes AceDoxyPC a potential interesting molecule to fight the neurological disturbances associated to Covid-19, which has recently been pointed out [25–27]. Beyond this PDX-containing LysoPC mimetic, we may extrapolate the use of other poxytrins-containing PC (named PCtrins).

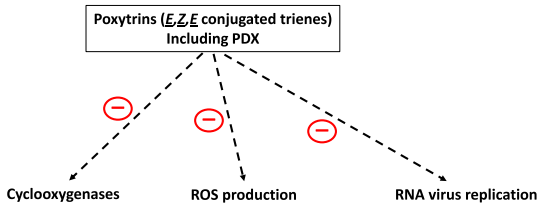

Fig. 3. Summary figure for potential bioactivities of poxytrins.

5. Conclusion

Poxytrins, as double lipoxygenase products of PUFA, might be physiological molecules active against some RNA virus replication, as well as against acute inflammation associated with those virus infection (Fig. 3). Altogether, it could then be considered that poxytrins, especially PDX, might be of interest in treatment of Covid-19.

Authors contributions

MG supervised the work done on poxytrins and contributed in writing the current manuscript.

NBH supervised the work done on AceDoxyPC and contributed in writing the current manuscript.

ML supervised all works and wrote the manuscript.

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References

[1] P. Chen, E. Véricel, M. Lagarde, M. Guichardant, Poxytrins, a class of oxygenated products from polyunsaturated fatty acids, potently inhibit blood platelet aggregation, Faseb. J. 25 (2011) 382–388, https://doi.org/10.1096/fj.10-161836.

[2] M. Lagarde, E. Véricel, M. Liu, P. Chen, M. Guichardant, Structure-function relationships of non-cyclic dioxygenase products from polyunsaturated fatty acids: poxytrins as a class of bioactive derivatives, Biochimie 107 (2014) 91–94, https://doi.org/10.1016/j.biochi.2014.09.008.

[3] P. Borgeat, B. Samuelsson, Transformation of arachidonic acid by rabbit polymorphonuclear leukocytes. Formation of a novel dihydroxyeicosatetraenoic acid, J. Biol. Chem. 254 (1979) 2643–2646.
[4] T. Shimizu, O. Rådmark, B. Samuelsson, Enzyme with dual lipoxygenase activities catalyzes leukotriene A4 synthesis from arachidonic acid, Proc. Natl. Acad. Sci. U. S. A 81 (1984) 689–693, https://doi.org/10.1073/pnas.81.3.689.
[5] P. Borgeat, B. Fruteau de Laclos, S. Picard, J. Drapeau, P. Vallerand, E.J. Corey, Studies on the mechanism of formation of the 5S, 12S-dihydroxy-6,8,10,14(E,Z,E,Z)-icosatetraenoic acid in leukocytes, Prostaglandins 23 (1982) 713–724, https://doi.org/10.1016/s0090-6980(82)80009-9.
[6] C.A. Dahinden, R.M. Clancy, T.E. Hugli, Stereospecificity of leukotriene B4 and structure-function relationships for chemotaxis of human neutrophils, J. Immunol. 133 (1984) 1477–1482.
[7] M. Croset, M. Lagarde, Stereospecific inhibition of PGH2-induced platelet aggregation by lipoxygenase products of icosaenoic acids, Biochem. Biophys. Res. Commun. 112 (1983) 878–883, https://doi.org/10.1016/0006-291x(83)91699-6.
[8] R.L. Maas, A.R. Brash, J.A. Oates, A second pathway of leukotriene biosynthesis in porcine leukocytes, Proc. Natl. Acad. Sci. U. S. A. 78 (1981) 5523–5527, https://doi.org/10.1073/pnas.78.9.5523.
[9] I.A. Butovich, A one-step method of 10,17-dihydro(pero)xydocosahexa-4Z,7Z,11E,13Z,15E,19Z-enoic acid synthesis by soybean lipoxygenase, J. Lipid Res. 47 (2006) 854–863, https://doi.org/10.1194/jlr.D500042-JLR200.
[10] P. Chen, B. Fenet, S. Michaud, N. Tomczyk, E. Véricel, M. Lagarde, M. Guichardant, Full characterization of PDX, a neuroprotectin/protectin D1 isomer, which inhibits blood platelet aggregation, FEBS Lett. 583 (2009) 3478–3484, https://doi.org/10.1016/j.febslet.2009.10.004.
[11] P. K Mukherjee, V.L. Marcheselli, C.N. Serhan, N.G. Bazan, Neuroprotectin D1: a docosahexaenoic acid-derived docosatriene protects human retinal pigment epithelial cells from oxidative stress, Proc. Natl. Acad. Sci. U. S. A. 10 (2004) 8491–8496, https://doi.org/10.1073/pnas.0402531101.
[12] M. Liu, P. Chen, E. Véricel, M. Lelli, L. Béguin, M. Lagarde, M. Guichardant, Characterization and biological effects of di-hydroxylated compounds deriving from the lipoxygenation of ALA, J. Lipid Res. 54 (2013) 2083–2094, https://doi.org/10.1194/jlr.M035139.
[13] C. Schneider, A.R. Brash, Stereospecificity of hydrogen abstraction in the conversion of arachidonic acid to 15r-HETE by aspirin-treated cyclooxygenase-2. Implications for the alignment of substrate in the active site, J. Biol. Chem. 275 (2000) 4743–4746, https://doi.org/10.1074/jbc.275.7.4743.
[14] M. Liu, T. Boussetta, K. Makni-Maalej, M. Fay, F. Driss, J. El-Benna, M. Lagarde, M. Guichardant, Protectin DX, a double lipoxygenase product of DHA, inhibits both ROS production in human neutrophils and cyclooxygenase activities, Lipids 49 (2014) 49–57, https://doi.org/10.1007/s11745-013-3863-6.
[15] M. Morita, K. Kuba, A. Ichikawa, M. Nakayama, J. Katahira, R. Iwamoto, T. Watanebe, S. Sakabe, T. Daidoji, S. Nakamura, A. Kadowaki, T. Ohto, H. Nakanishi, R. Taguchi, T. Nakaya, M. Murakami, Y. Yoneda, H. Arai, Y. Kawaoka, J.M. Penninger, M. Arita, Y. Imai, The lipid mediator protectin D1 inhibits influenza virus replication and improves severe influenza, Cell 153 (1) (2013 Mar 28) 112–125, https://doi.org/10.1016/j.cell.2013.02.027.
[16] Y. Imai, Role of omega-3 PUFA-derived mediators, the protectins, in influenza virus infection, Biochim. Biophys. Acta 1851 (2015) 496–502, https://doi.org/10.1016/j.bbalip.2015.01.006.
[17] W.J. Lukiw, J.-G. Cui, V.L. Marcheselli, M. Bodker, A. Botkjaer, K. Gotlinger, C.N. Serhan, N.G. Bazan, A role for docosahexaenoic acid-derived neuroprotectin D1 in neural Cell survival and alzheimer disease, J. Clin. Invest. 115 (2005), https://doi.org/10.1172/JCI25420, 2774-1783.
[18] C. Jouvène, B. Fourmaux, A. Géloën, L. Balas, T. Durand, M. Lagarde, M. Létisse, M. Guichardant, Ultra-performance liquid chromatography-mass spectrometry analysis of free and esterified oxygenated derivatives from docosahexaenoic acid in rat brain, Lipids 53 (1) (2018 Jan) 103–116, https://doi.org/10.1002/lipd.12006.
[19] F. Thies, C. Pillon, P. Moliere, M. Lagarde, J. Lecerf, Preferential incorporation of sn-2 lysoPC DHA over unesterified DHA in the young rat brain, Am. J. Physiol. 267 (1994) R1273–R1279, https://doi.org/10.1152/ajpregu.1994.267.5.R1273.
[20] L.N. Nguyen, D. Ma, G. Shui, P. Wong, A. Cazenave-Gassiot, X. Zhang, M.R. Wenk, E.L.K. Goh, D.L. Silver, Mfsd2a is a transporter for the essential omega-3 fatty acid docosahexaenoic acid, Nature 509 (2014) 503–506, https://doi.org/10.1038/nature13241.
[21] M. Croset, N. Brossard, A. Polette, M. Lagarde, Characterization of plasma unsaturated lysophosphatidylcholines in human and rat, Biochem. J. 345 (2000) 61–67.
[22] A. Polette, C. Deshayes, B. Chantegrel, M. Croset, J.M. Armstrong, M. Lagarde, Synthesis of acetyl,docosahexaenoyl-glycerophosphocholine and its characterization using nuclear magnetic resonance, Lipids 34 (1999) 1333–1337, https://doi.org/10.1007/s11745-999-0486-1.
[23] M. Hachem, A. Géloën, A. Lo Van, B. Foumaux, L. Fenart, F. Gosselet, P. Da Silva, G. Breton, M. Lagarde, M. Picq, N. Bernoud-Hubac, Efficient docosahexaenoic acid uptake by the brain from a structured phospholipid, Mol. Neurobiol. 53 (5) (2016 Jul) 3205–3215, https://doi.org/10.1007/s12035-015-9228-9.
[24] A. Lo Van, B. Fourmaux, M. Picq, M. Guichardant, M. Lagarde, N. Bernoud-Hubac, Synthesis and identification of AceDoxyPC, a protectin-containing structured phospholipid, using liquid chromatography/mass spectrometry, Lipids 52 (2017) 751–761, https://doi.org/10.1007/s11745-017-4280-z.
[25] S. Farhadian, L.R. Glick, C.B.F. Vogels, J. Thomas, J. Chiarella, A. Casanovas-Massana, J. Zhou, C. Odio, P. Vijayakumar, B. Geng, J. Fournier, S. Bermejo, J.R. Fauver, T. Alpert, A.L. Wyllie, C. Turcotte, M. Steinle, P. Paczkowski, C. Dela Cruz, C. Wilen, A.I. Ko, S. MacKay, N.D. Grubaugh, S. Spudich, L.A. Barakat, Acute encephalopathy with elevated CSF inflammatory markers as the initial presentation of COVID-19, BMC Neurol. 20 (2020) 2–5, https://doi.org/10.1186/s12883-020-01812-2.
[26] J.R. Berger, COVID-19 and the nervous system, J. Neurovirol. 26 (2020) 143–148, https://doi.org/10.1007/s13365-020-00840-5.
[27] A.A. Asadi-Pooya, L. Simani, Central nervous system manifestations of COVID-19: a systematic review, J. Neurol. Sci. 413 (2020) 1–4, https://doi.org/10.1016/j.jns.2020.116832.