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Review

Pathophysiology of the hepoxilins ☆

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ABSTRACT

There is increasing evidence from various scientific groups that hepoxilins represent novel inflammatory mediators. In vitro studies have shown that the hepoxilins cause mobilization of intracellular calcium in human neutrophils, cause plasma leakage, and potently stimulate chemotaxis of human neutrophils. In vivo, the hepoxilin pathway is activated in conditions of inflammation, e.g. after pathogen infection, in inflamed conditions (psoriasis, arthritis), and hepoxilins promote inflammatory hyperalgesia and allodynia. Although much work has demonstrated an effect of hepoxilins on neutrophils, the hepoxilin pathway has been demonstrated in a variety of tissues, including the lung, brain, pituitary, pancreatic islets, skin, etc. A genetic defect linked to a deficiency in hepoxilin formation has been described and believed to be responsible for the scaly skin observed in ichthyosis. Despite their biological and chemical instability, the involvement of the hepoxilin pathway in pathology has been demonstrated in vitro and in vivo through either isolation of the hepoxilins themselves (or their metabolites) or implied through the use of stable hepoxilin analogs. These analogs have additionally shown efficacy in animal models of lung fibrosis, cancer, thrombosis and diabetes. Research on these compounds has merely scratched the surface, but results published to date have suggested that the hepoxilin pathway is a distinct and novel pathway leading to inflammation and hepoxilin antagonists may provide the means of controlling early aspects of the acute inflammatory phase. This article is part of a Special Issue entitled "Oxygenated metabolism of PUFA: analysis and biological relevance".

1. Introduction

The hepoxilin pathway was discovered in the early 80s with the isolation of two compounds derived from the common intermediate, 12(S) hydroperoxy-eicosatetraenoic acid (12(S)-HPETE), a product of arachidonic acid (AA) formed through the 12S-lipoxygenase-induced reaction system present in the rat lung [1]. Scheme 1 provides an overview of the arachidonic acid cascade including formation of the hepoxilins and their metabolites.

Similar hepoxilin products derived from EPA [2] and DHA [3] have been reported. Analogous hepoxilin-like compounds derived from linoleic acid from skin have been reported [4]. The hepoxilin compounds possess both hydroxyl and epoxide groups formed from an intramolecular rearrangement of the hydroperoxy oxygen atoms of 12(S)-HPETE [5] (Scheme 2). Hence it was proposed that this was an enzymatic process, termed 'hepoxilin synthase' [6]. This was also shown to be a specific reaction as the 12(R)-HPETE was not a substrate for the isomerization reaction present in the rat pineal [7]. However

Abbreviations: AA, arachidonic acid; AMG9810, (2*E*)-*N*-(2,3-dihydro-1,4-benzodioxin-6-yl)-3-[4-(1,1-dimethylethyl)phenyl]-2-propenamide; Apaf-1, Apoptotic protease activating factor 1; BAPTA, 1,2-Bis(o-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid; CCCP, carbonyl cyanide *m*-chlorophenyl hydrazone; CDC, cinnamyl-3,4-dihydroxy-α-cyanocinnamate; DAG, diacylglycerol; DHA, docosahexaenoic acid; EH, epoxide hydrolase; eLOX3, epidermal lipoxygenase-3; EPA, eicosapentaenoic acid; fMLP, formyl-methionyl-leucyl-phenylalanine; GPCR, G-protein-coupled receptor; GppNHp, 5′-Guanylyl imidodiphosphate; GPx, glutathione peroxidase; GTP, Guanosine-5′-triphosphate; GTPγS, Guanosine-5′-O-[gamma-thio] triphosphate; HC030031, 1,2,3,6-Tetrahydro-1,3-dimethyl-N-[4-(1-methylethyl)phenyl]-2,6-dioxo-7H-purine-7-acetamide; HETE, hydroxyeicosatetraenoic acid; HODE, hydroxyoctadecadienoic acid; HP, hydroperoxy; HPETE, hydroperoxyeicosatetraenoic acid; HSP, heat shock protein; HX, hepoxilin; HXA_3-C, 11-Glutathionyl-hepoxilin A_3; IP3, inositol triphosphate; IP3R, inositol triphosphate receptor; K-562, chronic myelogenous leukemia human cell line; LOX, lipoxygenase; LTB_4, leukotriene B_4; NAT, n-Acetyl transferase; NECA, 5′-N-ethylcarboxamidoadenosine; PAF, platelet activating factor; PBT, proprietary bioactive therapeutics; PC, phosphatidyl choline; PE, phosphatidyl ethanolamine; PG, prostaglandin; PHGPx, phospholipid hydroperoxide gluthathione peroxidase; PPARγ, the Nuclear Peroxisome Proliferator Activator Receptor gamma isoform; ROS, reactive oxygen species; RVD, regulatory volume decrease; TCPO, 3,3,3-Trichloro-1,2-propene oxide; TRAP220, mediator of RNA polymerase II transcription subunit 1; TRPA1, transient receptor potential ankyrin 1 channel; TRPV1, transient receptor potential vanilloid 1 channel; TRX, trioxilin; U0126, 1,4-Diamino-2,3-dicyano-1,4-*bis*(2-aminophenylthio)butadiene, MEK Inhibitor VI; U937, cell line isolated from the histiocytic lymphoma of a 37-year old male patient; XOR, xanthine oxidoreductase

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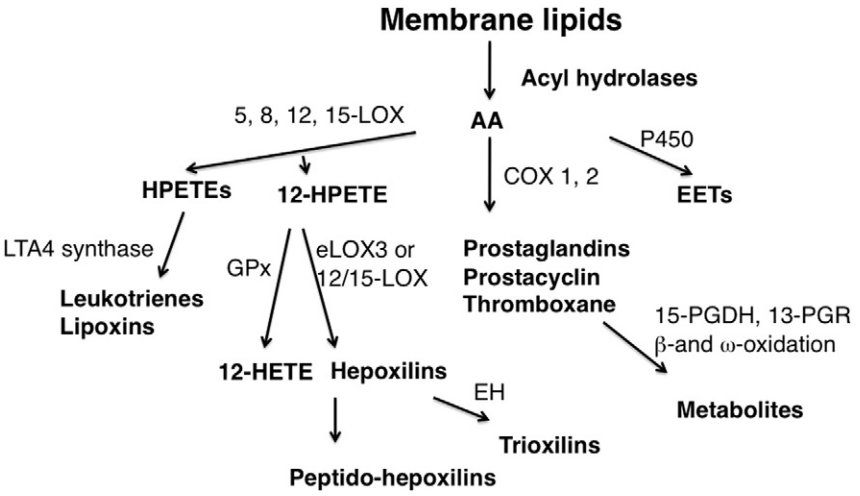


Scheme 1. Classical 'Arachidonic Acid Cascade' pathway showing diverse enzymatic mechanisms of oxidation of arachidonic acid. Several of these pathways have been described for EPA and DHA substrates.

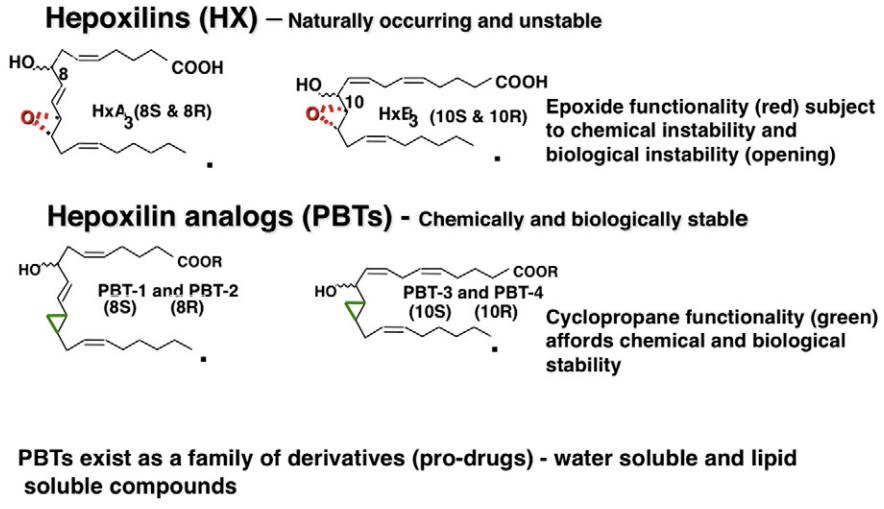
both 12(S) and 12(R)-HPETEs were converted into native (11S, 12S) and what we termed at the time, 'unnative' (11R, 12R) hepoxilins, when the reaction was catalyzed by heme-containing proteins in vitro [8]. Since then it has been shown that the 11R, 12R-enantiomer is uniquely formed in skin through a process initiated by 12(R)-lipoxygenase [9,11].

The enzymatic nature of the isomerization reaction to form the hepoxilins was later confirmed through two important findings 1) cloning experiments using rat insulinoma cells, RINm5F, demonstrated that the responsible isomerization activity was an intrinsic component of the 12(S)-lipoxygenase activity of the 12/15-lipoxygenase enzyme [10], and 2) demonstration that eLOX3, a protein which possesses no 12-LOX activity originally found in skin, acts in concert with 12(R)-LOX to convert the 12-LOX product, 12(R)-HPETE into 11R, 12R-hepoxilins [4,11,12] (see section on Control of hepoxilin/12-HETE formation).

Because the native hepoxilins are unstable chemically (degraded in acid environment) and biologically (degraded through cellular epoxide hydrolases) [13,14] to produce trihydroxy compounds (trioxilins) with much lesser (if any) biological activity, we developed stable analogs in the early 1990s to investigate the potential biological activity profile of the hepoxilins in vivo [15]. These analogs were designed to replace the unstable epoxide group with a stable cyclopropyl group (Scheme 2). In so doing, it was hoped that information could be obtained on the role of the endogenous hepoxilins. Indeed this review addresses the major findings from our group and those from several others showing the involvement of the hepoxilins in acute inflammation, the efficacy of the hepoxilin analogs as antagonists of the native hepoxilins, and to offer some insight as to the potential therapeutic interest generated by a recent finding that the hepoxilin analogs additionally cause some of their inhibitory effects in both the cancer animal model in vivo and on platelet aggregation in vitro through inhibition of the release of arachidonic acid, suggesting inhibition of an acyl hydrolase, likely a type of phospholipase A_2 [16].

2. Distribution of the hepoxilins

Studies in the early 1980s showed the presence of a unique transformation of 12(S)-HPETE in rat lung [1]. Two unstable products were isolated which were termed hepoxilin A_3 and hepoxilin B_3 (Scheme 2). Although the hepoxilins were further metabolized into their trihydroxy metabolites (trioxilins A_3 and B_3) through epoxide hydrolases present in the lung, hepoxilin synthetic/epoxide hydrolase activities were separated through ammonium sulfate fractionation of a low speed supernatant fraction of the lung homogenate [17]; thus, while 12(S)-HPETE and hepoxilins were formed by the 0–30% ammonium sulfate



Scheme 2. Structures of the native hepoxilins A_3 and B_3 and the corresponding synthetic stable analogs (PBTs). It should be noted that hepoxilin A3 is less stable than B3 due to the allylic nature of the epoxide.