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Review

Modulation of mitochondrial dysfunction and endoplasmic reticulum stress are key mechanisms for the wide-ranging actions of epoxy fatty acids and soluble epoxide hydrolase inhibitors

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ABSTRACT

The arachidonic acid cascade is arguably the most widely known biologic regulatory pathway. Decades after the seminal discoveries involving its cyclooxygenase and lipoxygenase branches, studies of this cascade remain an active area of research. The third and less widely known branch, the cytochrome P450 pathway leads to highly active oxygenated lipid mediators, epoxy fatty acids (EpFAs) and hydroxyeicosatetraenoic acids (HETEs), which are of similar potency to prostanoids and leukotrienes. Unlike the COX and LOX branches, no pharmaceuticals currently are marketed targeting the P450 branch. However, data support therapeutic benefits from modulating these regulatory lipid mediators. This is being approached by stabilizing or mimicking the EpFAs or even by altering the diet. These approaches lead to predominantly beneficial effects on a wide range of apparently unrelated states resulting in an enigma of how this small group of natural chemical mediators can have such diverse effects. EpFAs are degraded by soluble epoxide hydrolase (sEH) and stabilized by inhibiting this enzyme. In this review, we focus on interconnected aspects of reported mechanisms of action of EpFAs and inhibitors of soluble epoxide hydrolase (sEHI). The sEHI and EpFAs are commonly reported to maintain homeostasis under pathological conditions while remaining neutral under normal physiological conditions. Here we provide a conceptual framework for the unique and broad range of biological activities ascribed to epoxy fatty acids. We argue that their mechanism of action pivots on their ability to prevent mitochondrial dysfunction, to reduce subsequent ROS formation and to block resulting cellular signaling cascades, primarily the endoplasmic reticulum stress. By stabilizing the mitochondrial – ROS – ER stress axis, the range of activity of EpFAs and sEHI display an overlap with the disease conditions including diabetes, fibrosis, chronic pain, cardiovascular and neurodegenerative diseases, for which the above outlined mechanisms play key roles.

1. Introduction

Eicosanoids generated from cyclooxygenase (COX) and lipoxygenase (LOX) mediated metabolism of arachidonic acid (ARA) are well-known bioactive lipids that promote and maintain inflammatory signaling cascades. Efforts towards understanding the

Abbreviations: 4-PBA, 4-phenylbutyric acid; ARA, arachidonic acid; ATF6, activating transcription factor 6; BiP or Grp78, 78-kDa glucose regulated protein; COX, cyclooxygenase; CYP, cytochrome P450; DHA, docosahexaenoic acid; eIF2α, eukaryotic translation initiation factor 2 alpha; EPA, eicosapentaenoic acid; DHET, dihydroxyeicosatrienoic acid; EpDPE, epoxydocosapentaenoic acid; EET, epoxyeicosatrienoic acid; EpFA, epoxy fatty acid; ER, endoplasmic reticulum; HEPE, hydroxyeicosapentaenoic acid; HETE, hydroxyeicosatetraenoic acid; HFD, high fat diet; IRE1α, inositol-requiring enzyme 1 alpha; LOX, lipoxygenase; mEH, microsomal epoxide hydrolase; mPTP, mitochondrial permeability transition pore; $\Delta\Psi_m$, mitochondrial membrane potential; NSAID, non-steroidal anti-inflammatory drug; PERK, PKR-like ER-regulated kinase; RANK and RANKL, receptor activator of nuclear factor κ B and RANK ligand; ROS, reactive oxygen species; sEH, soluble epoxide hydrolase; sEHI, soluble epoxide hydrolase inhibitor; TPPU, 1-trifluoromethoxy-phenyl-3-(1-propionylpiperidin-4-yl) urea; *t*-TUCB, *trans*-4-{4-[3-(4-trifluoromethoxyphenyl)ureido]cyclohexyloxy}benzoic acid; TUPS, 1- trifluoromethoxy-phenyl-3 (1-methylsulfonyl)piperidin-4-yl) urea; UA-8, 13-(3-propylureido)tridec-8-enoic acid; UPR, unfolded protein response; XBP1, X-box binding protein 1

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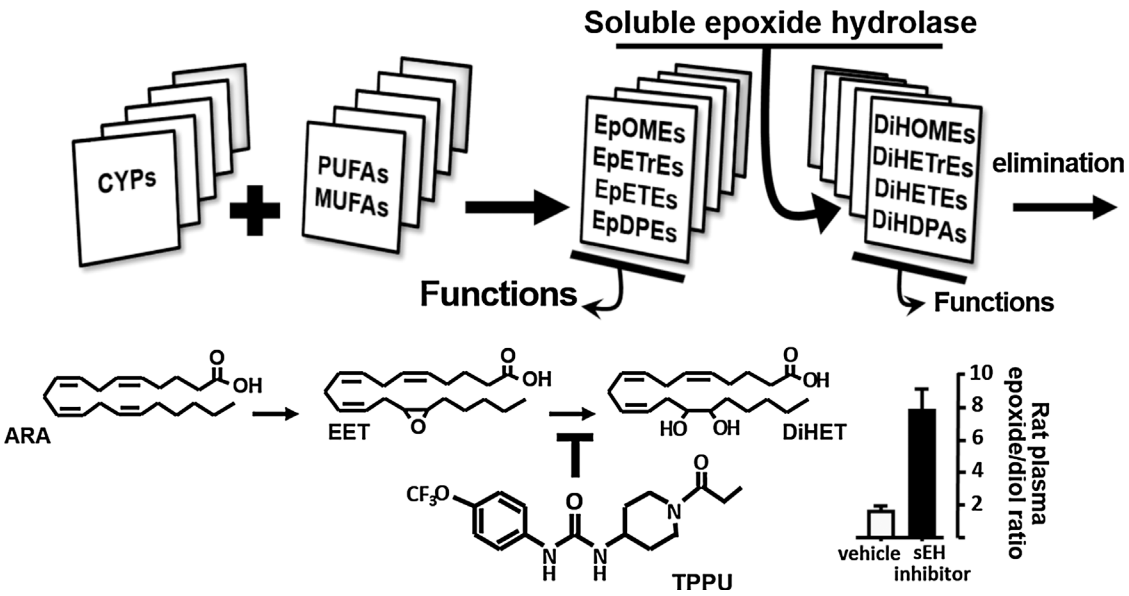

Fig. 1. Epoxides of unsaturated and largely polyunsaturated fatty acids (MUFAs and PUFAs) are the major anti-inflammatory and analgesic mediators of the P450 branch of the arachidonic acid cascade. These epoxides are made largely by cytochrome P450 enzymes and may be stored as phospholipids. The best studied are the EETs (EpETrEs), but epoxides of LA (EpOMEs), EPA (EpETEs), and DHA (EpDPEs) also are chemical mediators with epoxides of the ω-3 DHA or EDPs being particularly more? active. These epoxides are converted at varying rates but generally with high Vmax and low Km to the corresponding diols, for example EETs are converted by sEH to DHETs (DiHETrEs). These diols are generally less bioactive, tend to move out of cells, and are rapidly conjugated and excreted. DHETs and particularly the diols of linoleic acid have been shown to be pro-inflammatory at the high concentrations present in sepsis. Inhibitors of sEH such at TPPU stabilize epoxy fatty acids (EpFA) such as the EETs shown, to increase the lipid epoxide to diol ratios in the blood which are associated with reduced inflammation and pain.

fundamental characteristics of the potent and profound actions of these lipid metabolites in addition yielded a multitude of therapeutic approaches to quell inflammation and pain [1,2]. There is continued interest in therapeutically targeting the enzymes, receptors and metabolites within these two predominantly pro-inflammatory pathways, while the latest identified branch of the ARA cascade, known as the cytochrome P450 branch offers underexploited features and novel therapeutic targets [3].

The cytochrome P450 branch, yields potent ARA metabolites including hydroxyeicosatetraenoic acids (HETEs) and epoxyeicosatrienoic acids (EETs) [4]. Progress attained within the last decade strongly suggests that EETs and other EpFA are opposing counterparts to the largely pro-inflammatory prostanoids, leukotrienes and HETEs [5,6]. This P450 branch of the ARA cascade is attracting increasing attention both in fundamental regulatory biology and as a clinical target to treat a variety of diseases [7–9]. It is now clear that unsaturated free fatty acids in general are substrates for the cytochrome P450 s generating EpFA. There is considerable structural diversity among bioactive lipids bearing an epoxide functionality (Fig. 1). Together with the hepoxilins these molecules are classified as epoxy fatty acids (EpFAs).

1.1. Epoxide hydrolase mediated degradation rapidly regulates the titers of EpFAs

The epoxide moiety on an EpFA is dissimilar to the reactive epoxide toxins such as those from aflatoxin or polycyclic aromatic hydrocarbons in that it does not react with nucleophiles in biological milieu. In addition, the lipid backbones of EpFA do not intercalate into nucleic acids which would be critical for genotoxicity. The EpFAs are chemically stable at neutral pH although, consistent with their roles in cellular signaling, exhibit short *in vivo* half-lives due to rapid hydrolysis by epoxide hydration [10]. This property has been a stumbling block that impeded early progress in the field. The breakthrough was contingent on identification of epoxide hydrolase (EH) activity and recognition of the soluble epoxide hydrolase (sEH, *EPHX2,* E.C.3.3.2.10) as an enzyme that rapidly degrades EpFAs followed by tools to chemically and genetically knock out or induce the expression of this enzyme [8]. While mammals express several different epoxide hydrolases, sEH seems to be the common denominator for the inactivation of most, if not all EpFA species in most tissues. In tissue extracts, if sEH activity is blocked with selective inhibitors, much of EpFA degrading activity is eliminated [11]. Spector estimated that most of the degradation of EpFAs was due to sEH explaining why its inhibition increases titers of EpFAs *in vivo* [10]. However, these higher levels of EpFAs seem to be cleared from the system by degradation via alternate pathways limiting the EpFA titers achieved by sEHI. Therefore, degradation by sEH seems to be the major regulatory step that, with biosynthesis and release from phospholipid stores, adjusts the EpFA titers. In at least one exceptional case, brain microsomal EH (mEH, *EPHX1*, E.C.3.3.2.9) may contribute to degradation of EpFAs [12]. The mEH and sEH share a common structure and catalytic mechanism involving the formation and hydrolysis of a covalent intermediate [11]. Even though EpFAs are not among the preferred substrates for mEH, its expression in the brain is much higher than that of sEH suggesting that brain titers of EpFAs are co-regulated by both EHs. In several cases, the efficacy of sEH inhibitors have been enhanced by maintaining animals on a diet enriched with ω-3 and depleted in ω-6, suggesting that epoxides of DHA and EPA are more potent mediators in some systems than the corresponding EETs [13]. Additionally, the ω- terminal epoxides of EPA and DHA are turned over by sEH at much slower rate than most of the other regioisomers [14]. This *in vivo* stability may contribute to the biological activity of these EpFA and to the broad biological benefits of dietary ω-3 fatty acids.

The discovery of potent and selective inhibitors of sEH beginning early 2000 s resulted in broader interest towards EpFAs and the sEH [15]. Consequently, simultaneous progress was attained both in fundamental understanding of these bioactive lipid mediators and in therapeutic applications by way of inhibiting sEH. An increasing number of functions and activities of EpFAs and sEHI continue to be reported. With the use of potent sEH inhibitors, numerous groups published key observations that support clinical development of sEH inhibitors [7,16].