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The eosinophil chemoattractant 5-oxo-ETE and the OXE receptor

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Abstract

5-Oxo-ETE (5-oxo-6,8,11,14-eicosatetraenoic acid) is formed from the 5-lipoxygenase product 5-HETE (5S-hydroxy-6,8,11,14-eicosatetraenoic acid) by 5-hydroxyeicosanoid dehydrogenase (5-HEDH). The cofactor NADP⁺ is a limiting factor in the synthesis of 5-oxo-ETE because of its low concentrations in unperturbed cells. Activation of the respiratory burst in phagocytic cells, oxidative stress, and cell death all dramatically elevate both intracellular NADP⁺ levels and 5-oxo-ETE synthesis. 5-HEDH is widely expressed in inflammatory, structural, and tumor cells. Cells devoid of 5-lipoxygenase can synthesize 5-oxo-ETE by transcellular biosynthesis using inflammatory cell-derived 5-HETE. 5-Oxo-ETE is a chemoattractant for neutrophils, monocytes, and basophils and promotes the proliferation of tumor cells. However, its primary target appears to be the eosinophil, for which it is a highly potent chemoattractant. The actions of 5-oxo-ETE are mediated by the highly selective OXE receptor, which signals by activating various second messenger pathways through the release of the $\beta\gamma$ -dimer from G_{i/o} proteins to which it is coupled. Because of its potent effects on eosinophils, 5-oxo-ETE may be an important mediator in asthma, and, because of its proliferative effects, may also contribute to tumor progression. Selective OXE receptor antagonists, which are currently under development, could be useful therapeutic agents in asthma and other allergic diseases.

Keywords

5-Oxo-ETE; 5-Lipoxygenase; Eosinophils; Asthma; Chemoattractants; Inflammation

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1. Introduction

The 5-lipoxygenase (5-LO) pathway was discovered in 1976 with the identification of 5S-hydroxy-6E,8Z,11Z,14Z-eicosatetraenoic acid (5-HETE) as a product of arachidonic acid (AA) metabolism in human neutrophils [1] (Fig. 1). Three years later it was found that the precursor of 5-HETE, 5S-hydroperoxy-6E,8Z,11Z,14Z-eicosatetraenoic acid (5-HpETE), was also converted to the unstable epoxide intermediate leukotriene (LT) A₄ [2], which was in turn rapidly metabolized to two products: the dihydroxy compound LTB₄ [3] and the glutathione conjugate LTC₄ [4]. It was subsequently shown that 5-LO converts the 15-LO product 15-HpETE to the trihydroxytetraene lipoxin A₄ (LXA₄) [5]. In 1992 we identified a pathway for the oxidation of 5-HETE to 5-oxo-6E,8Z,11Z,14Z-eicosatetraenoic acid (5-oxo-ETE) by 5-hydroxyeicosanoid dehydrogenase (5-HEDH).

The formation of 5-LO products is tightly regulated and required initial activation of cPLA₂ by phosphorylation and its calcium-dependent translocation to the nuclear membrane [6], where it hydrolyzes membrane phospholipids to release AA, which binds to the nuclear membrane protein FLAP [7]. Like cPLA₂, 5-LO is activated by phosphorylation and, under the influence of calcium, translocates to the nuclear membrane where it oxidizes FLAP-bound AA to 5-HpETE and LTA₄ [8]. 5-HpETE is converted to 5-HETE and 5-oxo-ETE by the actions of a peroxidase and 5-HEDH, whereas LTA₄ is transformed to LTB₄ by LTA₄ hydrolase and to LTC₄, LTD₄, and LTE₄ by the sequential actions of LTC₄ synthase, γ -glutamyltransferase, and dipeptidase [9,10]. 5-LO is restricted principally to inflammatory cells, whereas the secondary enzymes are also found in other cell types. Therefore LTs can be synthesized both directly by inflammatory cells as well as by transcellular biosynthesis as a result of the conversion of leukocyte-derived LTA₄ to LTB₄ or LTC₄ by cells that do not express 5-LO [11].

Leukotrienes and 5-oxo-ETE are potent proinflammatory mediators, the actions of which are mediated by G protein-coupled receptors (GPCRs). The principle target of LTB₄ is the neutrophil [12], for which it is a potent chemoattractant, although it also activates other cells, including monocytes [13] and lymphocytes [14,15]. Its actions are mediated principally by the highly selective BLT₁ receptor [16]. Although LTB₄ can also activate the BLT₂ receptor, the primary ligand for the latter is the cyclooxygenase product 12-hydroxy-5,8,10-heptadecatrienoic acid (12-HHT) for which it has higher affinity [17]. The cysteinyl-LTs, principally LTD₄, are important mediators in asthma and have potent contractile effects on airway smooth muscle and promote its remodeling [18]. They also promote mucus secretion and vascular permeability. These actions are mediated by the cysLT₁ receptor for which LTD₄ has the highest affinity. The cysLT₂ receptor displays equal affinities for LTC₄ and LTD₄ and appears to play a role principally in the cardiovascular system [19]. There is also evidence for a selective receptor for LTE₄ [20], which has tentatively been identified as GPR99 [21]. 5-Oxo-ETE is a potent eosinophil chemoattractant, the actions of which are mediated by the OXE receptor.

In contrast to other 5-LO products, lipoxins have anti-inflammatory effects, and are important mediators in the resolution of inflammation. LXA₄ and 15-epi-LXA₄ (formed by aspirin-treated cox-2) inhibit neutrophil and eosinophil recruitment and stimulate monocyte

recruitment during the resolution phase following inflammation [22]. They act principally through the ALX (FPR2) receptor.

2. Biosynthesis and metabolism of 5-oxo-ETE

The discovery of a dehydrogenase that selectively oxidizes 5-HETE to 5-oxo-ETE stemmed from an observation that 6-*trans* isomers of LTB₄, but not LTB₄ itself, were converted to dihydro metabolites by human neutrophils [23]. The enzyme responsible for the reductase reaction was subsequently identified as a calmodulin-dependent eicosanoid ⁶-reductase that requires

NADPH as a cofactor [24]. Deuterium-labeling experiments indicated that the reduction was preceded by oxidation of the 5-hydroxyl group [23,24]. The failure of LTB₄ to be metabolized by this pathway suggested that a 6-*trans* double bond was required for recognition by the dehydrogenase catalyzing the initial step in this pathway. Since the biologically inactive 6-*trans* isomers of LTB₄ seemed unlikely to be the primary targets of a selective metabolic pathway we sought to identify the natural endogenous substrate for the dehydrogenase that initiated this process. 5-HETE was an obvious candidate, since, like 6-*trans* isomers of LTB₄, it contains a 5S-hydroxy group followed by a 6-*trans* double bond.

2.1. 5-Hydroxyeicosanoid dehydrogenase

We found that neutrophil microsomes rapidly oxidize 5-HETE to 5-oxo-ETE in the presence of NADP⁺ [25]. Further investigation of this enzyme in differentiated U937 cells revealed that it displays a low K_m for both 5-HETE (~500 nM) and NADP⁺ (K_m, 140 nM), for which it shows a 10,000-fold preference over NAD⁺ [26]. It is strongly inhibited by NADPH (K_i, 220 nM) so that its activity is dependent on the ratio of NADP⁺ to NADPH rather than on the absolute concentration of either pyridine nucleotide. The reaction appears to follow a ping-pong mechanism, in which the first product is released prior to binding of the second substrate.

5-HEDH is highly selective for 5S-HETE and is clearly distinct from various other eicosanoid dehydrogenases, most of which are found in the cytosol (Table 1). It displays little or no activity with other HETE positional isomers or 5R-HETE but does oxidize (albeit at a lower rate than 5-HETE) certain dihydroxyeicosanoids that contain a 5S-hydroxyl followed by a 6-*trans* double bond, including 6-*trans* isomers of LTB₄, 5S,12S-dihydroxy-6E,8Z,10E,14Z-eicosatetraenoic acid (5,12-diHETE), and 5S,15S-dihydroxy-6E,8Z,11Z,13E-eicosatetraenoic acid (5,15-diHETE) [25]. Additional structural features that are required for metabolism by 5-HEDH include a free carboxyl group (5-HETE methyl ester is a poor substrate) and a hydrophobic alkyl moiety at the ω-end of the molecule. 5-Oxo fatty acids with a carbon chain of less than 16 carbons or with a hydrophilic ω-hydroxyl group are very poor substrates [27].

5-HEDH has not yet been cloned, so no information is yet available about its mRNA or protein expression. However, 5-HEDH activity is widely expressed among different cell types, unlike 5-LO, which is largely restricted to inflammatory cells. In addition to inflammatory cells, 5-HEDH is found in endothelial, epithelial, and smooth muscle cells, as

well as in a variety of cancer cell lines (Table 2). This is reminiscent of LTA₄ hydrolase and LTC₄ synthase, which are responsible for the formation of LTB₄ and LTC₄, respectively, and are also more widely expressed than 5-LO [10].

2.2. Regulation of 5-oxo-ETE synthesis

Oxidation of 5-HETE to 5-oxo-ETE by 5-HEDH is dependent on the intracellular ratio of NADP⁺ to NADPH. In normal resting cells this ratio is very low, since cells maintain high levels of NADPH as a protective mechanism against oxidative stress. This is accomplished largely through the action of glucose-6-phosphate dehydrogenase, which oxidizes glucose-6-phosphate to 6-phosphogluconate using NADP⁺ as a cofactor (Fig. 2). This provides the NADPH necessary to return GSSG to its reduced state, following oxidation of GSH in the presence of H₂O₂ or other reactive oxygen species [28]. In phagocytic cells, high levels of NADPH are required to support the activity of NADPH oxidase, which produces superoxide to combat invading microorganisms.

Although neutrophil microsomes display high levels of 5-HEDH activity, when intact neutrophils are incubated with 5-HETE only small amounts of 5-oxo-ETE are formed [29]. This is primarily because the ratio of NADP⁺ to NADPH in these cells is too low to support appreciable oxidation of 5-HETE by 5-HEDH. Furthermore, a major competing metabolic pathway for 5-HETE, ω -oxidation to 5,20-diHETE, requires NADPH (Fig. 2). NADPH also promotes the further metabolism of 5-oxo-ETE to 5-oxo-20-HETE [30] and 6,7-dihydro-5-oxo-ETE [24]. In contrast, 5-oxo-ETE is the major 5-HETE metabolite formed by neutrophils in which the respiratory burst has been activated by phorbol myristate acetate (PMA) [31], resulting in a rapid increase in intracellular NADP⁺ levels [32]. 5-Oxo-ETE synthesis is also strongly stimulated by activation of the respiratory burst in other phagocytic cells, including eosinophils [33] and monocytes [34].

Oxidative stress also strongly stimulates the 5-HEDH-catalyzed oxidation of 5-HETE to 5-oxo-ETE [35]. H₂O₂ and t-butyl hydroperoxide promote 5-oxo-ETE formation in all of the cell types we investigated (Table 2), with the notable exception of neutrophils on which they have little effect [35], possibly due to the relatively low GSH and glutathione peroxidase levels in these cells [36]. Oxidative stress is particularly important in regulating the synthesis of 5-LO products in B lymphocytes. Although the individual components required for the synthesis of 5-LO products are highly expressed by these cells, they were reported to synthesize only small amounts of LTB₄ and 5-HETE after stimulation. In contrast, large amounts of these two products were formed in the presence of H₂O₂ or after depletion of intracellular GSH [37,38]. This is presumably related to the requirement for oxidation of Fe⁺⁺ to Fe⁺⁺⁺ to activate 5-LO [39]. We found that 5-oxo-ETE is a major product of AA metabolism in both tonsillar and CESS B cells when they are simulated under conditions of oxidative stress [40]. This is due to the combined effects of elevated 5-LO activity and NADP⁺ levels, resulting in the selective stimulation of 5-oxo-ETE synthesis. The time course for the effects of oxidative stress on 5-oxo-ETE synthesis in B lymphocytes as well as other cell types is highly correlated with dramatic increases in intracellular GSSG and NADP⁺ coupled with corresponding reductions in GSH and NADPH [26,40,41].

Another important factor in regulating 5-Oxo-ETE synthesis is cell death. Neutrophils spontaneously undergo apoptosis when cultured in the presence of FBS such that 75% of the cells are apoptotic after 24 h. This is accompanied by marked increases in the intracellular GSSG/GSH and NADP⁺/NADPH ratios as well as the ability of to synthesize 5-oxo-ETE from both 5-HETE and AA [32]. All of these changes can be inhibited by treatment of neutrophils with survival factors such as GM-CSF and forskolin as well as antioxidants such as deferoxamine and catalase, and the NADPH oxidase inhibitor diphenylene iodonium, suggesting that they are mediated by oxidative stress accompanying apoptosis. Cell death is also associated with increased 5-oxo-ETE synthesis in various cancer cell lines [42]. 4,7,10,13,16,19-Docosahexaenoic acid (DHA), which is known to have cytotoxic effects on cancer cells [43,44], increased the ability of PC3 (prostate), A-427 (lung), and MCF7 (breast) cancer cells to synthesize 5-oxo-ETE. Similar effects were observed with tamoxifen and MK-886. Increased synthesis of 5-oxo-ETE by dying cells could be important at sites of tissue damage and inflammation, where it could lead to the infiltration of inflammatory cells and prolongation of the inflammatory response.

2.3. Transcellular biosynthesis of 5-oxo-ETE

The wide distribution of 5-HEDH among different cell types compared to the more narrow distribution of 5-LO suggests that transcellular biosynthesis of 5-oxo-ETE could augment its levels *in vivo*, as has been shown to be true for both LTB₄ and LTC₄ [11]. To investigate this possibility we stimulated neutrophils in the presence of H₂O₂-treated PC3 prostate cancer cells and measured 5-oxo-ETE levels [42]. Ideal controls for this experiment would have been PC3 cells lacking 5-HEDH, but since this enzyme has not yet been cloned we inhibited its activity by depleting intracellular GSH with N-ethylmaleimide, thereby completely blocking the stimulatory effect of H₂O₂. Following stimulation with calcium ionophore in the presence of AA and H₂O₂ 5-oxo-ETE synthesis was 4 times higher in coin incubations of neutrophils with vehicle-treated PC3 cells compared to GSH-depleted PC3 cells, indicating that the major portion of 5-oxo-ETE synthesized under these conditions was due to oxidation of neutrophil-derived 5-HETE by 5-HEDH in PC3 cells. PC3 cells alone were unable to convert AA to appreciable amounts of 5-oxo-ETE under these conditions. It would seem likely that structural cells such as epithelial and endothelial cells could also contribute to 5-oxo-ETE production by transcellular biosynthesis from 5-HETE released by infiltrating cells at sites of inflammation (Fig. 3).

2.4. Other sources of 5-oxo-polyunsaturated fatty acids

Although AA is the most abundant precursor polyunsaturated fatty acid (PUFA) for the synthesis of 5-oxo-PUFA, a number of other PUFA can be converted to such products by a combination of 5-LO and 5-HEDH. Mead acid (5Z,8Z,11Z-eicosatrienoic acid), which accumulates in dietary essential fatty acid deficiency, is a good substrate for 5-LO, and is converted to 5S-hydroxy-6E,8Z,11Z-eicosatrienoic acid (5-HETrE) and LTA₃ [45,46]. Although LTA₃ is a substrate for LTC₄ synthase [45], being converted to LTC₃, it inhibits LTA hydrolase [47]. It is therefore not converted to significant amounts of LTB₃ and inhibits the formation of LTB₄ from AA. The major products of Mead acid metabolism by human neutrophils are 5-HETrE and 5-oxo-6,8,11-eicosatrienoic acid (5-oxo-ETrE) together with

smaller amounts of 6-trans isomers of LTB₃ [48]. As discussed in Section 6, 5-oxo-ETrE is a potent granulocyte chemoattractant.

Sebaleic acid (5Z,8Z-octadecadienoic acid) is secreted by human sebaceous glands and is the major PUFA found in human skin surface lipids [49]. Although it is not a precursor for leukotrienes because it has only two double bonds, it is still a substrate for 5-LO and is converted by human neutrophils to two main products: 5S-hydroxy-6,8-octadecadienoic acid (5-HODE) and 5-oxo-6,8-octadecadienoic acid (5-oxo-ODE), along with smaller amounts of their 20-hydroxy metabolites [50]. 5-Oxo-ODE has similar biological activities as 5-oxo-ETE (Section 6). Another precursor of 5-oxo-PUFA is the ω 3-PUFA 5,8,11,14,17-eicosapentaenoic acid (EPA), which is oxidized to 5S-hydroxy-6E,8Z,11Z,14Z,17Z-eicosapentaenoic acid (5-HEPE) and 5-oxo-6E,8Z,11Z,14Z,17Z-eicosapentaenoic acid (5-oxo-EPE) by human neutrophils [51]. Unlike 5-oxo-ETrE and 5-oxo-ODE, 5-oxo-EPE is less potent than 5-oxo-ETE.

2.5. Alternative pathways for 5-oxo-ETE formation

Although specific enzymatic pathways exist for the formation of most biologically active eicosanoids, these substances can also be formed non-enzymatically by lipid peroxidation [52]. This process normally results in the formation of multiple stereoisomers, most of which have limited biological activity. However, this is not true for 5-oxo-ETE, which has no chiral carbons. Lipid peroxidation initiated by treatment of red cell ghosts with t-butyl hydroperoxide resulted in the formation of substantial amounts of 5-oxo-ETE bound to membrane phospholipids [53]. Similarly, treatment of plasmenyl glycerophosphocholine containing AA with the free radical initiator 2,20-azobis(2-amidinopropane) hydrochloride or with Fe⁺⁺ or Cu⁺⁺ led to the formation of esterified 5-HETE and 5-oxo-ETE [54]. The 5-oxo-ETE formed under the above conditions was presumably derived by loss of H₂O from 5-HpETE.

5-Oxo-ETE is formed by murine peritoneal macrophages following incubation with the calcium ionophore A23187 [55] in spite of the fact that these cells do not contain any appreciable 5-HEDH activity, since they were unable to convert 5-HETE to 5-oxo-ETE [56]. Instead, 5-oxo-ETE was formed directly from 5-HpETE in the presence of a heme-containing cytosolic protein. Since this activity was heat- and protease-resistant it was probably the heme group itself that was responsible for this reaction, as heme compounds are known to promote the dehydration of hydroperoxy fatty acids [57]. 5-Oxo-ETE is also formed after incubation of murine mast cell cytosolic fractions with AA, presumably by a similar mechanism [58]. As well as undergoing nonenzymatic dehydration, 5-HpETE can be converted enzymatically to 5-oxo-ETE by the action of the dioxin-inducible human cytochrome P450 CYP2S1 [59].

Isomers of 5-oxo-ETE have also been reported. Small amounts of 8-trans-5-oxo-ETE are formed as a result of nonenzymatic decomposition of 5-oxo-ETE. Another isomer of 5-oxo-ETE, 5-oxo-7E,9E,11Z,14Z-eicosatetraenoic acid, has been detected following incubation of high concentrations of AA with neutrophils in the presence of A23187 [58]. This compound is formed by the non-enzymatic decomposition of LTA₄ with retention of the triene

chromophore and is quite distinct from the 5-oxo-EETE that is the subject of the present review.

2.6. Metabolism of 5-oxo-EETE

5-Oxo-EETE is metabolized by a number of pathways, depending on the cell type involved. In most cases, this is accompanied by substantial losses in biological activity. Since 5-HEDH is a reversible enzyme, it can reduce 5-oxo-EETE to 5S-HETE in the presence of NADPH. However, the $V_{\max}$ for the ketoreductase reaction is about 8 times lower than that for the dehydrogenase reaction, thus favoring the formation of 5-oxo-EETE [26]. 5-Oxo-EETE, like 5-HETE [60], is incorporated into cellular lipids in neutrophils [61] and various other cells.

The major pathway for metabolism of 5-oxo-EETE by human neutrophils is ω -oxidation, resulting in the formation of 5-oxo-20-HETE [30]. This appears to be catalyzed by CYP4F3 (LTB₄ 20-hydroxylase), which also oxidizes 5-HETE to 5,20-diHETE [62]. 5-Oxo-EETE is also metabolized by ω -oxidation in murine macrophages [55]. However, unlike human neutrophils, these cells do not possess significant 20-hydroxylase activity, and instead convert 5-oxo-EETE to 18- and 19- hydroxy metabolites. Like human neutrophils [24], murine macrophages possess eicosanoid δ^6 -reductase activity, resulting in the formation of 6,7-dihydro metabolites (18- and 19- hydroxy derivatives of 5-oxo-8,11,14-eicosatrienoic acid) [55]. All of the above pathways result in dramatically reduced biological activity.

5-Oxo-EETE can also be metabolized by different lipoxygenases to 5-oxo-HETEs. Eosinophils, which are rich in 15-LO activity [63], convert it to 5-oxo-15-HETE (Fig. 3), which has biological effects similar to 5-oxo-EETE, although it is somewhat less potent (Section 3.1). This product is also formed from AA by soybean 15-LO [64] as well as from 5,15-diHETE by neutrophil 5-HEDH [25]. 5-Oxo-EETE can also be metabolized by 12-LO. Platelets highly express this enzyme along with 5-HEDH and convert neutrophil-derived 5-HETE to 5-oxo-12-HETE by transcellular metabolism [65] (Fig. 3). Interestingly, the latter product has antagonist properties (Section 9). Both 5-oxo-EETE and 5-oxo-12-HETE inhibited platelet aggregation, but only at μ molar concentrations [65].

A variety of eicosanoids containing α , β -unsaturated keto groups undergo Michael addition reactions with GSH catalyzed by GSH transferases [66]. 5-Oxo-EETE can be converted non-enzymatically at alkaline pH to a 9-glutathionyl conjugate (i.e. a 1,6-Michael addition product) [67], whereas GSH transferases catalyze 1,4-Michael addition reactions to give 7-glutathionyl conjugates [68]. None of these glutathione conjugates possesses significant biological activity. In contrast, LTC₄ synthase from murine macrophages and human platelets converts 5-oxo-EETE to a different 7-glutathionyl conjugate with a distinct stereochemical configuration that is biologically active. This compound, named FOG₇, had chemoattractant effects on human neutrophils and eosinophils and stimulated actin polymerization (but not calcium mobilization) in these cells [67]. However, because of its considerable structural differences from 5-oxo-EETE it is unlikely that these actions are mediated by the 5-oxo-EETE receptor.

3. Effects of 5-oxo-ETE on different cell types

The discovery of an enzyme that selectively converts 5-HETE to 5-oxo-ETE raised the possibility that the latter compound may have some biological function. 5-HETE had previously been shown to be a weak activator of human neutrophils, acting by a receptor distinct from those for other lipid mediators [69] and it seemed possible that 5-oxo-ETE could be the true ligand for this receptor. This was confirmed by the finding that 5-oxo-ETE is about 100 times more potent than 5-HETE in mobilizing calcium in neutrophils [25]. Furthermore, 5-oxo-ETE completely desensitized neutrophils to 5-HETE, and vice versa, but did not affect their response to other lipid mediators. Further studies revealed that 5-oxo-ETE has similar effects on other inflammatory cells, most notably eosinophils, which may be its primary target.

3.1. Eosinophils

The first evidence that 5-oxoeicosanoids have chemoattractant effects on human eosinophils resulted from the finding by Schröder's group that eosinophils convert AA to a product that they termed eosinophil chemotactic lipid (ECL) [70]. ECL was distinct from LTB₄, 5,15-diHETE, and 8,15-diHETE and was a potent chemoattractant for human eosinophils, unlike LTB₄, which displays little chemotactic activity for these cells, in contrast to its potent effects on guinea pig eosinophils [71]. Incubation of AA with soybean 15-LO led to the formation of a substance with similar properties that was identified as 5-oxo-15-HETE [64]. 5-Oxo-15-HETE was subsequently shown to be formed from both 5-oxo-ETE and 5,15-diHETE by eosinophils [63], which are well known to highly express 15-LO [72] as well as 5-HEDH [33]. Eosinophils also convert arachidonic acid to 5-oxo-ETE [33], which is also a potent chemoattractant for these cells [33,63,73]. However, the major 5-LO products released by eosinophils are cysteinyl-LTs. There is some discrepancy in the literature about the relative potencies of 5-oxo-ETE and 5-oxo-15-HETE in inducing this response, with Schröder's group finding them equipotent [63], whereas we [33] and others [73] find 5-oxo-ETE to be more potent.

5-Oxo-ETE and 5-oxo-15-HETE are the only 5-LO products with appreciable eosinophil chemotactic activity and among all of the lipid mediators to which it has been compared, including platelet-activating factor (PAF), 5-oxo-ETE has the most powerful effects [33,73]. Although leukotrienes B₄, C₄, D₄, and E₄ display very modest direct effects on eosinophil migration, these are negligible compared to that of 5-oxo-ETE [33]. The only other eicosanoid with substantial eosinophil chemotactic activity is PGD₂ [74,75] and some of its degradation products [76,77], which act through the DP₂ receptor. Although low concentrations of 5-oxo-ETE and PGD₂ have similar effects, the maximal response to 5-oxo-ETE is about three times higher [74]. It is possible that PGD₂ and 5-oxo-ETE could act in concert with one another to induce and maintain eosinophil infiltration. PGD₂ is rapidly released from mast cells following exposure to allergen [78], and may act acutely to initiate eosinophil infiltration, whereas 5-oxo-ETE may be released over a prolonged period of time by inflammatory and structural cells exposed to oxidative stress and undergoing apoptosis. With respect to CC chemokines 5-oxo-ETE is slightly more potent than RANTES as an

eosinophil chemoattractant and about one-tenth as potent as eotaxin. However, as with PGD₂, the maximal response to 5-oxo-ETE is substantially greater [79].

Although cell migration in response to chemoattractants is critical for the tissue infiltration of eosinophils, they must also cross the endothelium and basement membrane, a process that is dependent on proteases. 5-Oxo-ETE is a potent stimulator of eosinophil migration through Matrigel basement membranes [80]. This effect is due not only to its chemoattractant effects but also to its ability to induce the release and expression of MMP-9 as well as the expression of the urokinase-type plasminogen activator receptor (uPAR) [81,82]. These activities promote the degradation of matrix components, enabling eosinophils to migrate across the basement membrane. This response to 5-oxo-ETE is inhibited by antibodies against MMP-9 and uPAR as well as by a metalloproteinase inhibitor [80]. 5-Oxo-ETE also strongly promotes the migration of eosinophils through endothelial cell monolayers [83].

In vivo, 5-oxo-ETE promotes tissue infiltration of eosinophils following intradermal injection in humans [84]. Elevated numbers of these cells were observed 6 and 24 h after administration in both asthmatic and non-allergic subjects. However, asthmatics responded more strongly, with about three times the numbers of eosinophils compared to healthy controls.

5-Oxo-ETE also elicits various rapid responses in eosinophils, including increased levels of cytosolic calcium and polymerized actin [63,85,86], which reach maximal levels after only 5 seconds [86]. Over a somewhat longer period of time (several minutes), increases in the surface expression of CD11b and the shedding of L-selectin are also observed [86]. Interestingly, LTB₄ is equipotent with 5-oxo-ETE in inducing calcium mobilization and slightly more potent in stimulating CD11b expression [86] in spite of its lack of appreciable eosinophil chemotactic activity, as discussed above. In contrast, 5-oxo-ETE is a much stronger inducer of actin polymerization than LTB₄, suggesting that this response is more closely related to cell migration than calcium mobilization or CD11b expression. 5-Oxo-ETE is also much more potent than LTB₄ in initiating L-selectin shedding [86]. It also induces surface expression of the leukocyte activation marker CD69 and appears to mediate the effect of PAF and IL-5 on this response, in contrast to LTB₄, which has little or no effect [87]. The respiratory burst [85] and the release of granule enzymes, including β -glucuronidase, eosinophil peroxidase and arylsulfatase [73] are also triggered by 5-oxo-ETE, although the degranulation response is rather modest in the absence of priming by cytokines.

A variety of both lipid and peptide mediators are involved in regulating the course of inflammation and 5-oxo-ETE acts synergistically with many of them, including other lipid mediators, chemokines, and cytokines. Low concentrations of 5-oxo-ETE enhance eosinophil migration in response to PAF [33] and dramatically increase degranulation in response to PAF, LTB₄, and C5a [73]. Furthermore, threshold concentrations of eotaxin and RANTES shift the concentration-response curve for 5-oxo-ETE-induced eosinophil chemotaxis to the left by about one order of magnitude [79]. In the above experiments both chemoattractants were present only in the bottom chambers of the microchemotaxis chambers. When added to the top chambers, 5-oxo-ETE inhibited eosinophil migration

towards PGD₂, but enhanced migration towards eotaxin, which were present only in the bottom chambers [88]. In contrast, when the situation was reversed, PGD₂ in the top chambers augmented eosinophil migration to 5-oxo-ETE when it was present only in the bottom chambers. These findings suggested to the authors that these different chemoattractants, because of their production at different times and locations during the inflammatory response, could coordinate the tissue infiltration of eosinophils.

Certain cytokines also enhance the responsiveness to eosinophils to 5-oxo-ETE. Priming with GM-CSF dramatically increased eosinophil degranulation in response to 5-oxo-ETE [73], whereas pretreatment with IL-5 augmented their 5-oxo-ETE-induced migration through Matrigel [80].

5-Oxo-ETE-induced eosinophil activation can also be downregulated by certain ligands. A nicotinic receptor agonist inhibited the 5-oxo-ETE-induced release of MMP-9 from eosinophils as well as their migration through Matrigel [89]. The DHA metabolite protectin D₁ reduced 5-oxo-ETE-induced eosinophil migration, CD11b expression, and L-selectin shedding [90]. However, these inhibitory effects were not specific for 5-oxo-ETE, as responses to eotaxin were reduced in a similar fashion in both of the above cases.

3.2. Neutrophils

As noted above, the biological activity of 5-oxo-ETE was first discovered in studies with neutrophils [91,92] and these cells are often used to investigate the effects of 5-oxoeicosanoids because they are more readily available than eosinophils. The responses of neutrophils to 5-oxo-ETE are very similar to those of eosinophils, including chemotaxis [91], calcium mobilization [91,92], actin polymerization [93,94], and CD11b expression [94]. 5-Oxo-ETE also promotes neutrophil aggregation [95], adherence, and increased surface expression of CD11c [94]. In contrast to eosinophils, unprimed neutrophils generate little [93] or no [96] superoxide in response to 5-oxo-ETE. However, a robust response was observed after pretreatment of these cells with GM-CSF [96] or in the presence of low concentrations of PAF [95]. Similarly, 5-oxo-ETE alone had a relatively modest effect on the release of granule enzymes (β -glucuronidase and lysozyme) from neutrophils, but induced a much stronger response following priming with either TNF- α [92] or G-CSF [96] or when added together with PAF or ATP [95]. Intradermal injection of 5-oxo-ETE in humans elicits neutrophil infiltration into the skin, but to a lesser extent than eosinophils [84].

In general, neutrophils do not appear to respond quite as strongly as eosinophils to 5-oxo-ETE [73,85], possibly because they express lower numbers of 5-oxo-ETE receptors [97] (see Sect. 9). Moreover, 5-oxo-ETE is only about one-tenth as potent as LTB₄ in activating neutrophils. Since LTB₄ and 5-oxo-ETE would both be produced following activation of 5-LO, the physiological significance of 5-oxo-ETE as a neutrophil chemoattractant is not entirely clear. However, it is possible that 5-oxo-ETE may play a role in certain situations. For example, neutrophils can become desensitized to LTB₄ after *in vivo* exposure [98]. It is conceivable that this could occur in inflammation and under these circumstances 5-oxo-ETE could play an increasingly important role in eliciting neutrophil infiltration. There may also be time-related differences in LTB₄ and 5-oxo-ETE levels at inflammatory sites. We found that although higher concentrations of LTB₄ were initially formed after stimulation of

neutrophils with A23187, its levels declined rapidly due to its metabolism to ω -oxidation products. In contrast, 5-oxo-ETE rose continuously after prolonged culture in the presence of 0.5% FBS to reach maximal levels after 24 h, which were almost 15 times higher than those of LTB₄ [32]. Thus it is possible that 5-oxo-ETE could play a role as a neutrophil chemoattractant under conditions of prolonged or chronic inflammation.

A very interesting paper has recently appeared in which an *in vivo* role for 5-oxo-ETE and the OXE receptor in leukocyte infiltration in zebrafish has been documented [99]. In this model, external injury exposes underlying cells to hypotonic medium resulting in cell swelling, which triggers the infiltration of leukocytes (principally neutrophils and some macrophages). Down-regulation of various potential mediators of this response using morpholinos and enzyme inhibitors revealed that initial steps were the activation of cPLA₂ and 5-LO. Although LTB₄ has chemoattractant effects on zebrafish leukocytes, it did not appear to be involved in damage-induced leukocyte infiltration, since this response was unaffected by inhibition or down-regulation of LTA₄ hydrolase. 5-Oxo-ETE was also shown to induce leukocyte infiltration and this was blocked by down-regulation of the OXE receptor with a morpholino, the selectivity of which was confirmed by its lack of effect on the response to LTB₄. This morpholino also blocked damage-induced leukocyte infiltration, demonstrating a role for 5-oxo-ETE and the OXE receptor. It was proposed that this process was initiated by the activation of cPLA₂ in response to cell swelling, followed by the synthesis of 5-HETE and its conversion to 5-oxo-ETE under the influence of oxidative stress resulting from DUOX (dual oxidase) activation [99].

3.3. Basophils

5-Oxo-ETE is a potent chemoattractant for basophils, inducing a response similar to PGD₂ and slightly less than that to eotaxin [100,101]. It is also a potent stimulator of basophil migration through a Matrigel basement membrane, inducing responses similar to those of RANTES, IL-8, and PAF [102]. As with eosinophils, basophil transmigration was dependent on the release of MMP-9, as it could be blocked by a selective inhibitor. However, in contrast to eosinophils, these responses were only observed with basophils that had been primed with IL-3. 5-Oxo-ETE is also a potent inducer of shape change in basophils [101], but has only modest effects on other responses, including calcium mobilization [100,101], and cell surface expression of CD11b [100,103] and CD203c [103]. 5-Oxo-ETE does not induce degranulation of basophils, as assessed by histamine release [100,101,103] and cell surface expression of the degranulation marker CD63 [103].

3.4. Monocytes

Monocytes are also activated by 5-oxo-ETE. It is a potent inducer of migration of these cells, but its efficacy, although similar to LTB₄, is only about half that of other monocyte chemoattractants such as fMLP and MCP-1 [104]. However, there are strong synergistic interactions between 5-oxo-ETE and both MCP-1 and MCP-3, resulting in markedly enhanced cell migration. 5-Oxo-ETE also induces actin polymerization in monocytes to nearly the same degree as MCP-1, but does not stimulate calcium mobilization [104].

Another potentially important effect of 5-oxo-ETE on monocytes is its ability to induce the release of GM-CSF [105]. Addition of 5-oxo-ETE to eosinophils in the presence of small numbers of monocytes leads to markedly enhanced eosinophil survival. Conditioned medium from 5-oxo-ETE-treated monocytes has a similar effect, which can be blocked by an antibody against GM-CSF. Analysis of the medium by ELISA confirmed the stimulatory effect of 5-oxo-ETE on GM-CSF secretion [105]. This could be an important mechanism whereby 5-oxo-ETE could increase the survival of eosinophils and possibly other infiltrating cells at inflammatory sites.

3.5. Tumor cells

Reports of a possible link between high fat diets and cancer [106] prompted Ghosh and Myers to examine the effects of AA and its metabolites on prostate tumor cell proliferation and survival. They found that AA promotes the proliferation of PC3 and LNCaP prostate cancer cells. This effect was not prevented by inhibitors of cox, 12-LO, or cytochrome P450 but was blocked by both 5-LO inhibitors (nordihydroguaiaretic acid and AA-861) and a FLAP inhibitor (MK-886) [107]. The proliferative effect of AA was mimicked by 5-oxo-ETE and to a lesser extent by its precursor 5-HETE, but not by LTB₄, LTC₄, or LTD₄. Furthermore, both MK-886 and AA-861 induced apoptosis in prostate cancer cells in the absence of exogenous AA and this could be prevented by treatment with 5-oxo-ETE and 5-HETE, but not by a variety of other AA metabolites, suggesting that 5-oxo-ETE is an endogenous regulator of prostate tumor cell growth [108]. 5-Oxo-ETE also inhibited selenium-induced apoptosis in prostate cancer cells [109] and increased the rates of proliferation of cancer cells derived from various other tissues, including breast (MDA-MB-231 and MCF7 cells) and ovary (SKOV3 cells) [110]. The antiapoptotic and proliferative effects of 5-oxo-ETE appear to be mediated by the OXE receptor, which is expressed in prostate tumor cells but not in normal prostate epithelial cells [111]. Blocking its expression with siRNA reduced the viability of PC3 cells, lending further support for a role for 5-oxo-ETE as an endogenous regulator of prostate tumor cell growth [111]. The proliferative effect of 5-oxo-ETE on these cells appears to be mediated through PLC- β , release of diacyl glycerol, and activation of PKC- ϵ [112,113].

4. The 5-oxo-ETE receptor

The high degree of selectivity for stimulation of granulocytes by 5-oxoeicosanoids, the desensitization with similar ligands (5-oxo-ETE, 5-oxo-15-HETE and 5-HETE) and the lack of desensitization with other agents such as the structurally related LTB₄ [91] suggested that 5-oxo-ETE acts by its own dedicated receptor. The actions of 5-oxo-ETE can be blocked by pertussis toxin, indicative of a GPCR [30,93,96]. Binding studies in neutrophils were initially complicated by the esterification of 5-oxo-ETE into membrane lipids, but this problem was circumvented by using the fatty acyl CoA synthetase inhibitor triacsin C [61]. Under these conditions radiolabeled 5-oxo-ETE bound to neutrophil membranes with a K_d of about 4 nM. It could be displaced by higher concentrations of related ligands (5-oxo-ETE > 5-HETE >> 5,15-diHETE) but not by other mediators such as LTB₄, PAF, or various peptide chemoattractants, indicating that the 5-oxo-ETE receptor is highly selective.

The receptor for 5-oxo-ETE was independently cloned by 3 groups. Using a BLAST nucleotide search for sequences with homology to GPCRs, Hosoi and coworkers identified a candidate intronless sequence localized to chromosome 2p21 that was predicted to code for a 423 amino acid protein, which they tentatively named TG1019 [114]. They used constructs in which TG1019 was fused to $G\alpha_i$, $G\alpha_s$, or $G\alpha_q$ to screen a library of potential ligands for their ability to stimulate GTP γ S binding. By far the most potent ligand was 5-oxo-ETE (5-oxo-ETE $\gg$ 5-HpETE $\gg$ 5S-HETE = 5R-HETE). Takeda and coworkers independently identified the 5-oxo-ETE receptor, codenamed hGPCR48 [115] from a group of intronless orphan GPCRs they had identified from database searches [116]. Using a GTP γ S binding assay similar to that described above they identified 5-oxo-ETE as the ligand for this receptor, with an EC_{50} of about 5 nM [115]. hGPCR48 is identical to TG1019. Finally, Jones and coworkers identified an orphan GPCR termed R527 using a data mining approach and screened for its ligand by measuring calcium mobilization in HEK293 cells transfected with both R527 and $G\alpha_{16}$ [97]. Of the potential ligands tested 5-oxo-ETE was the most potent (EC_{50} ~100 nM) in stimulating calcium mobilization. 5-HpETE was about 100 times less potent whereas 5-HETE had only a very modest effect in this assay. R527 appears to be a truncated form of TG1019, as it lacks the first 39 amino acids of the amino terminus. However, this did not appear to affect its biological activity.

The 5-oxo-ETE receptor (TG1019/hGPCR48/R527) has been designated as the OXE receptor by the IUPHAR Nomenclature Committee for Leukotriene and Lipoxin Receptors [117]. The corresponding gene is *OXER1*. The OXE receptor is highly expressed at the mRNA level in peripheral leukocytes, spleen, lung, liver and kidney [114]. Consistent with its biological effects on leukocytes, OXE mRNA expression is highest in eosinophils > neutrophils > bronchoalveolar macrophages [97]. It is also expressed in basophils [100,101] and monocytes [101] as well as various cancer cell lines [110,111] and an adrenocortical cell line [118].

Among receptors for 5-LO products, OXE is most closely related to the cysteinyl-LT receptors cysLT₁ and cysLT₂ (Fig. 4). When compared to GPCRs in general the OXE receptor bears the closest homology to the hydroxyl-carboxylic acid receptors HCA1 (39% identity and 52% similarity over 290 amino acids), HCA2 (40% identity and 58% similarity over 294 amino acids), and HCA3 (41% identity and 59% similarity over 294 amino acids) (Fig. 4). The endogenous ligand for the HCA1 receptor, previously known as GPR81, is lactate [119]. The ligand for HCA2 (previously known as GPR109A) is β -D-hydroxybutyric acid, whereas that for HCA3 (previously known as GPR109B) is the fatty acid β -oxidation intermediate 3-hydroxyoctanoic acid [119]. Both HCA2 and HCA3 also bind nicotinic acid. The OXE receptor is also related to GPR31 (34% identity and 52% similarity over 284 amino acids), which has recently been reported to be a high affinity (K_d ~5 nM) receptor for 12S-HETE [120].

Orthologs of *OXER1* exist in a variety of mammalian species (Fig. 5), including primates, cats, dogs, cows, sheep, elephants, opossums, pandas, and ferrets, as well as in some species of fish. An OXE ortholog has been identified in zebrafish that is activated by 5-oxo-ETE and is involved in regulating leukocyte recruitment in this species [99] (see Section 3.2). The gene for this *oxer1* ortholog is known as *gpr81-4*. However, there is unfortunately no

OXER1 ortholog in rodents. The absence of the OXE receptor in mice has been a significant impediment to our understanding of the physiological and pathophysiological roles of 5-oxo-ETE and the OXE receptor because of the abundance of murine animal models.

5. OXE receptor-independent effects of 5-oxo-ETE

5.1. Effects of 5-oxo-ETE on human airway smooth muscle

5-Oxo-ETE has a relaxant effect on human bronchial smooth muscle pre-contracted with methacholine [121]. This effect was prevented by the BK_{Ca} channel inhibitor iberiotoxin and was accompanied by hyperpolarization of the resting membrane potential. Furthermore, reconstituted BK_{Ca} channels from human bronchial smooth muscle were activated by 5-oxo-ETE. Interestingly, these effects were not blocked by pertussis toxin [121], which as far as is presently known, blocks all responses mediated by the OXE receptor. Moreover, OXE receptor mRNA was undetectable in human airway smooth muscle cells [97], suggesting that it does not mediate the relaxant effect of 5-oxo-ETE.

5.2. Effects of 5-oxo-ETE in rodents

Despite their lack of an *OXER1* ortholog, there are some reports of biological activities of 5-oxo-ETE in rodents, raising the possibility that an additional 5-oxo-ETE receptor may exist in these, and possibly other, species. We found that intratracheal administration of 5-oxo-ETE to Brown Norway rats resulted in infiltration of eosinophils into the lungs [122]. The intensity of this response was similar to that induced by PAF and slightly greater than that to LTB₄. This was confirmed in a later study, in which we found that the degree of pulmonary eosinophilia elicited by 5-oxo-ETE was slightly greater than that to PGD₂ and about the same as to the selective DP₂ receptor agonist 15R-methyl-PGD₂ [123]. The response to 5-oxo-ETE was not mediated by either BLT₁ or PAF receptors, since doses of selective antagonists (LY255283 and WEB 2170, respectively) that blocked the responses to LTB₄ and PAF had no effect on 5-oxo-ETE-induced pulmonary eosinophilia [122].

Guinea pigs also respond to 5-oxo-ETE, which was found to rapidly reduce the volumes of jejunal crypt epithelial cells [124]. 5-Oxo-ETE was more potent than any of a variety of other agonists tested in inducing this response, which could be blocked by PKC inhibitors. In contrast to crypt cells, jejunal villus cell volume was unaffected. 5-Oxo-ETE stimulated the contraction of guinea pig airway smooth muscle [125], in contrast to its relaxant effect on human airway smooth muscle. This effect was mediated by the release of TXA₂, since it could be blocked by both the cyclooxygenase inhibitor indomethacin as well as by the selective TP receptor antagonist SQ-29548. The mechanism appeared involve sensitization of myofilaments to calcium through activation of the Rho kinase pathway.

5-Oxo-ETE increased the expression of steroidogenic acute regulatory protein (StAR) and steroidogenesis in mouse MA-10 Leydig tumor cells and had similar effects on human adrenocortical H295R cells [118]. Although H295R cells express the OXE receptor, it is possible that the steroidogenic effects of 5-oxo-ETE may be mediated by a different receptor in both cell types.

6. Structure-activity relationships

We have conducted extensive studies on the structural requirements for activation of the OXE receptor, primarily using calcium mobilization and CD11b expression in neutrophils and actin polymerization in eosinophils. This receptor is highly selective for 5-oxo-EETE, with relatively minor structural modifications resulting in dramatic losses in biological activity. Starting from the carboxylic acid end of the molecule, a free carboxyl group is important for activity, as methylation reduces potency by 20-fold [30]. Thus it is unlikely that esterified 5-oxo-EETE, which can be formed during lipid peroxidation [53], would be capable of activating the OXE receptor. The 5-oxo-^{6E,8Z}-dienone system is clearly critical for biological activity. Reduction of the 5-oxo group to a hydroxyl group (i.e. 5-HETE) reduces potency by at least 100-fold in both neutrophils [91] and transfected cells [114]. Reduction of the 6,7-*trans* double bond (i.e. 5-oxo-8Z,11Z,14Z-eicosatrienoic acid) results in an even larger 1000-fold reduction in potency [24]. The 8,9-*cis* double bond is also important, since the monoene 5-oxo-6E-eicosenoic acid has little activity [48]. However, reversing the configuration of this double bond (8-*trans*-5-oxo-EETE) is tolerated to some extent, as it results in only a 6-fold reduction in potency. The remaining ^{11Z,14Z} double bonds appear to be less important, as 5-oxo-6E,8Z-eicosadienoic acid is approximately one-tenth as potent as 5-oxo-EETE [48]. Moreover, the 5-oxo-^{6E,8Z}-diene 5-oxo-ODE [50], which is formed from sebaleic acid, and the 5-oxo-^{6E,8Z,11Z}-triene 5-oxo-ETrE, which is formed from Mead acid, are equipotent with 5-oxo-EETE. However, introduction of an additional ^{17Z} double bond, as in the EPA metabolite 5-oxo-HEPE, reduced potency by about 10-fold [51].

In addition to the 5-oxo-^{6E,8Z}-dienone system, activation of the OXE receptor requires the presence of a hydrophobic tail on the agonist molecule. We investigated the activities of a series of even chain length 5-oxo-^{6E,8Z}-dienones ranging from 12 to 20 carbons and found that a minimum of 18 carbons is required for appreciable agonist activity [48]. Furthermore, the presence of an ω -hydroxyl group (5-oxo-20-HETE [30]) dramatically reduced potency. Interestingly, a hydroxyl group in the 15-position is better tolerated than one in the 20-position. 5-Oxo-15-HETE is an OXE receptor agonist, even though it is less potent than 5-oxo-EETE [33,73]. We recently prepared this compound (5-oxo-15S-hydroxy-6E,8Z,11Z,13E-eicosatetraenoic acid) by total chemical synthesis and found it to be about one-tenth as potent as 5-oxo-EETE in stimulating calcium mobilization in neutrophils [126]. In contrast, 5-oxo-12-HETE does not mobilize calcium in these cells, but instead blocks the response to 5-oxo-EETE [65], possibly because of the proximity of the 12-hydroxyl group to the 5-oxo-^{6,8}-diene portion of the molecule. It may be that the 15-hydroxyl group is a sufficient distance from both the hydrophobic ω -end of the molecule and the 5-oxo-^{6,8}-diene segment to permit appreciable agonist activity. The regions of 5-oxo-EETE that are critical for its biological activity are shown in Fig. 6.

7. Intracellular signalling

The actions of 5-oxo-EETE appear to be dependent almost exclusively on G_{i/o} proteins as they can be blocked by pertussis toxin [30,93,96]. Unlike the receptors for a number of other chemoattractants, the OXE receptor is not coupled to G_q [114,127]. One of the earliest

responses to 5-oxo-EETE is mobilization of intracellular calcium, which is blocked by the phospholipase C (PLC) inhibitor U73122, indicating that it is mediated by the PLC-dependent hydrolysis of phosphatidylinositol 4,5-bisphosphate, resulting in the release of inositol trisphosphate from the cell membrane [128]. (Fig. 7). Activation of PLC also results in the liberation of diacyl glycerol, which is known to stimulate both conventional and novel isoforms of PKC. PLC- δ has been reported to mediate some of the actions of 5-oxo-EETE in eosinophils [82]. The proliferative effect of 5-oxo-EETE on prostate cancer cells appears to be mediated by PKC- ϵ , and this can be blocked by the PLC inhibitor U73122, consistent with the involvement of diacyl glycerol in this response [113]. PKC- ζ has also been implicated in 5-oxo-EETE-induced eosinophil migration [82], but in this case, activation of PLC may not be involved, as this isoform is not activated by calcium or diacyl glycerol.

The OXE receptor also signals through phosphoinositide-3 kinase (PI3K), since increased levels of phosphatidylinositol (3,4,5)-trisphosphate were observed following treatment of neutrophils with 5-oxo-EETE [93]. This was confirmed in experiments with CHO cells transfected with the OXE receptor, in which 5-oxo-EETE induced the phosphorylation of Akt, which was blocked by LY294002, a PI3K inhibitor [128]. Both LY294002 and U73122 blocked 5-oxo-EETE-induced migration of these cells, suggesting that activation of both PI3K and PLC are required for 5-oxo-EETE-induced cell migration in this model [128].

ERK-1 and ERK-2 are involved in mediating some of the actions of 5-oxo-EETE in both neutrophils [96] and eosinophils [73,82] and phosphorylation of both ERK isoforms has been demonstrated in these cells as well as in OXE receptor-transfected CHO cells [128]. 5-Oxo-EETE also induces phosphorylation of cPLA₂ accompanied the release of AA in neutrophils [96,129], presumably mediated by ERK, which is known to activate cPLA₂ by phosphorylation at ser-505 [130]. p38 MAP kinase is also rapidly phosphorylated in eosinophils in response to 5-oxo-EETE [82,88], and p38 inhibitors have been shown to block 5-oxo-EETE-induced migration through Matrigel [82].

Since the OXE receptor is coupled to G_{i/o} proteins 5-oxo-EETE would be expected to inhibit adenylyl cyclase, and this has been demonstrated in both transfected cells [114] and human neutrophils [131]. However, the importance of inhibition of cAMP formation in the biological actions of 5-oxo-EETE is not yet clear. A very interesting paper was recently published by Kostenis's group [131], who used a biased OXE receptor ligand (Gue1654; see Section 9) to demonstrate that inhibition of adenylyl cyclase by 5-oxo-EETE is mediated by the α_i G protein subunit, whereas all of the other responses to 5-oxo-EETE are mediated by the $\beta\gamma$ dimer (Fig. 7).

8. Potential involvement of 5-oxo-EETE in different diseases

8.1. Asthma and other eosinophilic disorders

The lack of an OXER1 ortholog in rodents has been an obstacle to our understanding of the pathophysiological role of 5-oxo-EETE. In large part because of the unavailability of murine disease models there have been no studies directly investigating the role of 5-oxo-EETE and the OXE receptor in any specific disease. However, the high expression of this receptor on eosinophils and basophils and the potent chemoattractant effects of 5-oxo-EETE, resulting in

their migration through the endothelium and basement membrane (Sections 3.1 and 3.3), suggest that these molecules may play an important role in diseases such as asthma and allergic rhinitis. Eosinophils play a key role in asthma [132], as they release a number of proteins that cause tissue damage, including major basic protein, eosinophil cationic protein, and eosinophil peroxidase [133]. They are also a source of various cytokines and growth factors, including TGF- β , and appear to play a role in tissue remodelling [134]. Eosinophils are an abundant source of cysteinyl-LTs, which are potent bronchoconstrictors, stimulate mucus secretion and induce the release of proinflammatory cytokines [18]. Although the role of basophils in asthma is not as well established as that for eosinophils, these cells could also be involved because of their ability to release IL-4, IL-13, and cysteinyl-LTs [135].

In addition to its direct effects on eosinophils, 5-oxo-ETE has synergistic interactions with other eosinophil chemoattractants as discussed in Section 3.1. Of particular interest is its relationship with GM-CSF, which is released from monocytes in response to 5-oxo-ETE and in turn promotes eosinophil survival and primes these cells to respond much more strongly to 5-oxo-ETE. Furthermore, GM-CSF can stimulate the formation of 5-LO products at multiple levels [136–138] and could thereby potentially increase the production of 5-oxo-ETE. Because of the requirement for elevated levels of NADP⁺, the synthesis of 5-oxo-ETE is favored under conditions of oxidative stress, as occur at inflammatory sites populated by phagocytic cells in which NADPH has been activated and under conditions of cells stress such as the zebrafish model discussed in Section 3.2. In these circumstances, 5-oxo-ETE could promote prolonged inflammation and could potentially contribute to conditions such as severe asthma.

8.2. Cancer

There is evidence that 5-oxo-ETE could play a role in cancer because of its proliferative effect on cancer cells from various sources (Section 3.5). Both prostate cancer-derived cell lines and prostate cancer tissue from patients contain high levels of the OXE receptor at both protein and mRNA levels [111,113]. 5-LO expression is elevated in prostate tumors [139] and both 5-HETE [108,140] and 5-oxo-ETE [112] were detected in tissue culture media from prostate cancer cell lines. As noted in Section 2.3, PC3 cells highly express 5-HEDH and, in coinubation experiments, convert leukocyte-derived 5-HETE to 5-oxo-ETE [42]. Large numbers of infiltrating inflammatory cells, including both neutrophils [141] and eosinophils [142] are found in tumors. Although these cells may in some cases have antitumor effects, they can also promote tumor progression due to the release of reactive oxygen species, cytokines, and chemoattractants [143,144]. Neutrophils, macrophages, and eosinophils express high levels of 5-LO and 5-HEDH, and could both synthesize 5-oxo-ETE directly as well as provide 5-HETE to tumor cells for transcellular synthesis of 5-oxo-ETE, which could be promoted by reactive oxygen species released by infiltrating phagocytic cells. 5-Oxo-ETE produced within tumors could both promote tumor cell growth and induce further infiltration of inflammatory cells, including eosinophils, which are very prominent within some tumors [142]. There is evidence that dying tumor cells release a substance that is a chemoattractant for eosinophils [142], for which 5-oxo-ETE would be an excellent candidate. Thus the effectiveness of cytotoxic agents could be compromised by increased production of 5-oxo-ETE, especially in the presence of infiltrating inflammatory cells.

8.3. Other diseases

There is relatively little information about the levels of 5-oxo-EETE in body fluids or tissues. In a study on human subjects with severe pulmonary hypertension it was found that the levels of 5-oxo-EETE and 5-HETE in lung tissue were about 3 times higher than in control subjects [145]. Low levels of LTB₄ were also detected, but these were unchanged. Interestingly, the levels of 5-oxo-EETE and 5-HETE were dramatically lower in hypertensive patients who had been treated with prostacyclin. It is possible that 5-oxo-EETE could contribute to lung inflammation in this condition. Another study focused on the effects of ischemia on eicosanoid formation in rat brain [146]. The levels of 5-HETE in brain tissue increased 10 times following ischemia, whereas those of 5-oxo-EETE were 50 times higher and twice as high as those of 5-HETE. In contrast, LTB₄ was below the detection limit. These results raise the possibility that 5-oxo-EETE could promote neutrophil infiltration and inflammation in ischemic brain.

9. OXE receptor antagonists

Because of the potent effects of 5-oxo-EETE on eosinophils and their potential roles in asthma and allergy and possibly in cancer, the OXE receptor is an attractive therapeutic target. Selective OXE receptor antagonists should also be very useful tools in determining the pathophysiological roles of 5-oxo-EETE in disease models in animals expressing this receptor, considering the unavailability of genetic models in mice.

The first compounds reported to have antagonist activity against the OXE receptor are two 5-oxo-EETE metabolites formed by platelets [65]. These substances, which were identified as 5-oxo-12-HETE (Fig. 8) and 5-oxo-8-trans-12-HETE, blocked 5-oxo-EETE-induced calcium mobilization in neutrophils with IC₅₀ values of 0.5 μ M and 2.5 μ M, respectively, while themselves having no effect on intracellular calcium levels. Because of their instability and susceptibility to metabolism these compounds would not be good drug candidates, but these findings did demonstrate the feasibility of searching for antagonists with more suitable properties.

Certain long-chain polyunsaturated fatty acids have also been reported to act as OXE receptor antagonists. 8,11,14-Eicosatrienoic acid, 11,14,17-eicosatrienoic acid, EPA, and DHA have all been reported to block the stimulatory effects of 5-oxo-EETE on GTP γ S binding to a fused OXE receptor-G α_i fusion protein [114] with IC₅₀ values between 2 and 6 μ M. However, in practice, these substances would not be useful as OXE receptor antagonists because they have a variety of other effects including, in some cases, the activation of inflammatory cells [147]. Moreover, EPA and DHA can be converted to anti-inflammatory resolvins and protectins that have a variety of independent effects of their own [148].

There has been some recent progress in the development of synthetic OXE receptor antagonists. Blättermann and coworkers screened a selected library for compounds with antagonist activity by assessing their effects on 5-oxo-EETE-induced calcium mobilization in HEK293 cells transfected with both the OXE receptor and G α_{16} [131]. Two compounds with modest antagonist activity were initially identified. The structural requirements for binding of these compounds to the OXE receptor were then predicted by computer modeling

based on the X-ray structure of the chemokine receptor CXCR4. A virtual screen based on the above compounds using the ZINC database (zinc.docking.org) led to the identification of several candidates that were screened biologically. One of these (Gue1654) was found to block 5-oxo-ETE-induced calcium mobilization, shape change, and chemotaxis in neutrophils and eosinophils. Gue1654 appears to be selective for the OXE receptor, as it did not block the effects of a variety of other chemoattractants. In contrast, Gue1654 failed to prevent the inhibitory effect of 5-oxo-ETE on adenylyl cyclase in neutrophils, indicating that it is a biased ligand for this receptor. Experiments with cells transfected with different G protein subunits revealed that Gue1654 blocks $G\beta\gamma$ -mediated signaling by the OXE receptor but does not affect $G\alpha_i$ -mediated signaling [131].

We have used a structure-based approach to design synthetic OXE receptor antagonists. Based on our prior structure-activity studies (Section 6) we knew that the first 5 carbons of 5-oxo-ETE (i.e. 5-oxovalerate), an adjacent diene system, and the terminal hydrophobic region of the molecule are required for activation of the receptor (Fig. 6). We reasoned that incorporation of these portions of 5-oxo-ETE onto a rigid indole scaffold might result in a conformationally restricted compound with high affinity for a conformation of the OXE receptor that is not associated with its activation. We tested a series of indoles containing both hexyl and 5-oxovalerate groups in different positions for their abilities to block 5-oxo-ETE-induced calcium mobilization in human neutrophils. Among these we identified one compound with appreciable antagonist activity (IC_{50} , $\sim 1.6 \mu M$), which contained a 5-oxovalerate group in the 1-position and a hexyl group in the 2-position [149]. Similar compounds with the substituents in other positions were much less active, suggesting that the antagonist mimics a hairpin conformation of 5-oxo-ETE. None of the compounds tested had any agonist activity. We investigated the effects of a variety of structural modifications and found that antagonist potency was increased by about 4-fold (IC_{50} , 400 nM) by the presence of a chloro substituent in the 6-position (compound VG-346; Fig. 8) [149]. Additional structural modification of VG-346 led to more potent OXE receptor antagonists with IC_{50} values of 25–30 nM (manuscript in preparation). None of these antagonists have agonist activity and they are selective for the OXE receptor, as they do not affect the responses to a variety of other chemoattractants. In addition to calcium mobilization, they also block 5-oxo-ETE-induced chemotaxis and actin polymerization in neutrophils and eosinophils.

10. Conclusions

5-Oxo-ETE is member of the 5-LO group of lipid mediators among which it the only one, apart from its less potent metabolites 5-oxo-15-HETE and FOG₇, that has potent chemoattractant effects on eosinophils. The OXE receptor, which mediates its biological effects, is most highly expressed on eosinophils and is also expressed on a variety of other inflammatory cells for which it is a chemoattractant, including basophils, neutrophils, monocytes, and macrophages. Because of the involvement of eosinophils in asthma and allergy the OXE receptor is an attractive drug target for these diseases. 5-Oxo-ETE is formed by the highly selective enzyme 5-HEDH, and its synthesis is tightly regulated by the availability of the cofactor NADP⁺. Intracellular NADP⁺ concentrations are elevated following activation of the respiratory burst in phagocytic cells, in the presence of oxidative

stress, and in cell death, all of which are associated with inflammation. Such an environment should favor the synthesis of 5-oxo-ETE, which could prolong this condition by promoting the further infiltration of inflammatory cells and increasing their survival through the release of GM-CSF. The OXE receptor is also expressed on some cancer cell lines in which 5-oxo-ETE was found to induce cell proliferation, suggesting that it could be a potential target for anti-cancer drugs. Although OXE orthologs exist in many species, it is not present in rodents, which has hampered research into the pathophysiological role of 5-oxo-ETE in animal models. However, potent and selective OXE receptor antagonists are currently under development, and these should be very useful both in defining the role of 5-oxo-ETE and potentially as therapeutic agents in asthma and other allergic diseases and possibly in the treatment of cancer.

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Abbreviations

12-HHT	12-hydroxy-5Z,8E,10E-heptadecatrienoic acid
5,12-diHETE	5S,12S-dihydroxy-6E,8Z,10E,14Z-eicosatetraenoic acid
5,15-diHETE	5S,15S-dihydroxy-6E,8Z,11Z,13E-eicosatetraenoic acid
5-HEDH	5-hydroxyeicosanoid dehydrogenase
5-HEPE	5S-hydroxy-6E,8Z,11Z,14Z,17Z-eicosapentaenoic acid
5-HETE	5S-hydroxy-6E,8Z,11Z,14Z-eicosatetraenoic acid
5-HETrE	5S-hydroxy-6E,8Z,11Z-eicosatrienoic acid
5-HODE	5S-hydroxy-6E,8Z-octadecadienoic acid
5-HpETE	5S-hydroperoxy-6E,8Z,11Z,14Z-eicosatetraenoic acid
5-LO	5-lipoxygenase
5-oxo-12-HETE	5-oxo-12S-hydroxy-6E,8Z,10E,14Z-eicosatetraenoic acid
5-oxo-15-HETE	5-oxo-15S-hydroxy-6E,8Z,11Z,13E-eicosatetraenoic acid
5-oxo-20-HETE	5-oxo-20-hydroxy-6E,8Z,11Z,14Z-eicosatetraenoic acid
5-oxo-EPE	5-oxo-6E,8Z,11Z,14Z,17Z-eicosapentaenoic acid
5-oxo-ETE	5-oxo-6E,8Z,11Z,14Z-eicosatetraenoic acid

5-oxo-ETrE	5-oxo-6,8,11-eicosatrienoic acid
5-oxo-ODE	5-oxo-6E,8Z-octadecadienoic acid
DHA	4Z,7Z,10Z,13Z,16Z,19Z-docosahexaenoic acid
ECL	eosinophil chemotactic lipid
EPA	5Z,8Z,11Z,14Z,17Z-eicosapentaenoic acid
FOG₇	5-oxo-7-glutathionyl-8,11,14-eicosatrienoic acid
GPCR	G protein-coupled receptor
LT	leukotriene
LXA₄	lipoxin A ₄
Mead acid	5Z,8Z,11Z-eicosatrienoic acid
PAF	platelet-activating
PI3K	phosphoinositide-3 kinase
PLC	phospholipase C
PMA	phorbol myristate acetate
PUFA	polyunsaturated fatty acid
Sebaleic acid	5Z,8Z-octadecadienoic acid
StAR	steroidogenic acute regulatory protein
uPAR	urokinase-type plasminogen activator receptor

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Figure 1.
Biosynthesis of 5-LO products from AA.

Figure 2. Regulation of intracellular NADP⁺ levels and 5-oxo-EETE synthesis

NADP⁺ is generated by the glutathione redox pathway in which GSH peroxidase (GPx) reduces H₂O₂ and oxidizes GSH to GSSG, which is reduced back to GSH by glutathione reductase (GRed) accompanied by the generation of NADP⁺. NADPH oxidase (NOX) also produces NADP⁺ when it reduces oxygen to superoxide. 5-Oxo-EETE synthesis is promoted by elevated levels of NADP⁺, which is also reduced back to NADPH by the pentose phosphate pathway, initiated by the action of glucose-6-phosphate dehydrogenase (G6Pdh) on glucose-6-phosphate (G6P). Both 5-oxo-EETE and 5-HETE are metabolized by ω -oxidation in the presence of NADPH.

Figure 3. Biosynthesis and metabolism of 5-oxo-EET in different cell types

Neutrophils and eosinophils can synthesize and metabolize 5-oxo-EET from endogenous AA, whereas PC3 prostate cancer cells can synthesize 5-oxo-EET from inflammatory cell-derived 5-HETE by transcellular biosynthesis. Platelets can also form 5-oxo-EET in this way, but convert it to 12-hydroxy metabolites by the action of 12-LO. LTC₄ synthase converts 5-oxo-EET to FOG₇ (broken line) although this has not yet been demonstrated to occur specifically in eosinophils.

Figure 4. Dendrogram showing the relationship of the OXE receptor to other eicosanoid receptors (large circles) as well as to other closely related receptors (small circles)
 This was constructed using the facilities at www.phylogeny.fr [156].

Figure 5. Homology between the human OXE receptor protein sequence and those of OXE receptors from other selected species

Identical and different amino acids are shown in red and cyan, respectively, whereas missing and additional amino acids are shown in black and blue, respectively. Sequence comparisons were made using BLAST on the NCBI website.

Figure 6.
Features of 5-oxo-EET that are important for activation of the OXE receptor, based on structure-activity relationships in human neutrophils and eosinophils.

Figure 7. Intracellular signaling in response to activation of the OXE receptor

The inhibitory effect of 5-oxo-ETE on adenylyl cyclase (AC) is mediated by the α_i G protein subunit, whereas all other effects are mediated by the $\beta\gamma$ dimer.

Figure 8. 5-Oxo-EETE antagonists

Structures of 5-oxo-EETE, 5-oxo-12-HETE, the structure-based antagonist VG-346, and the biased antagonist Gue1654. The concentration-response curve for inhibition of 5-oxo-EETE-induced calcium mobilization in neutrophils by VG-346 is shown on the right.

Table 1

Comparison of 5-HEDH to other eicosanoid dehydrogenases

Enzyme	Principal substrates	Cofactor	Subcellular localization	Reference
5-HEDH	5-HETE	NADP ⁺	Microsomes	[25]
12-HEDH	LTB ₄ , 12-HETE	NAD ⁺	Microsomes	[150]
LTB ₄ 12-hydroxy dehydrogenase ^a	LTB ₄	NADP ⁺	Cytosol	[151]
15-PGDH	PGs E ₂ , F _{2α} and I ₂ , 15- HETE, 12-HHT	NAD ⁺	Cytosol	[152]
11-HydroxyTXB ₂ dehydrogenase	TXB ₂	NAD ⁺	Cytosol	[153]

^a Also displays 15-keto-PG 13-reductase activity in the presence of either NADH or NADPH [152]

Table 2

Cells that express 5-HEDH activity and its regulation by conditions that effect intracellular NADP⁺ levels. All of the cells listed are of human origin.

Cell type	Regulation	Reference
Neutrophils	Respiratory burst; Cell death	[25,32,35]
Monocytes	Respiratory burst; Oxidative stress	[34,35]
Eosinophils	Respiratory burst	[33]
B-lymphocytes	Oxidative stress	[40]
Dendritic cells ^a		[154]
Platelets	Oxidative stress	[35,65]
Airway epithelial cells ^b	Oxidative stress	[41]
Airway smooth muscle cells	Oxidative stress	[41]
Vascular endothelial cells ^c		[155]
Keratinocytes	Oxidative stress	[50]
Cancer cell lines:		
U937 (monocytic)	Oxidative stress	[26]
HL-60 (neutrophilic)	Oxidative stress	[26]
CESS (B lymphocyte)	Oxidative stress	[40]
PC3 (prostate)	Oxidative stress; cell death	[42]
A549 (airway epithelial)	Oxidative stress	[41]
A-427 (lung)	Cell death	[42]
MCF7 (breast)	Cell death	[42]
T84 (colon)		[41]

^aDifferentiated from circulating monocytes

^bNormal human bronchial epithelial cells; BEAS 2B cells

^cHuman aortic endothelial cells