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Cytochrome P450 oxidase 2C inhibition adds to ω-3 long-chain polyunsaturated fatty acids protection against retinal and choroidal neovascularization

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Abstract

Objective—Pathological ocular neovascularization is a major cause of blindness. Increased dietary intake of ω-3 long-chain polyunsaturated fatty acids (LCPUFA) reduces retinal and choroidal neovascularization, but ω-3 LCPUFA metabolites of a major metabolizing pathway, cytochrome P450 oxidase (CYP) 2C, promote ocular pathological angiogenesis. We hypothesized that inhibition of CYP2C activity will add to the protective effects of ω-3 LCPUFA on neovascular eye diseases.

Approach and Results—The mouse models of oxygen-induced retinopathy (OIR) and laser-induced choroidal neovascularization (CNV) were used to investigate pathological angiogenesis in the retina and choroid respectively. The plasma levels of ω-3 LCPUFA metabolites of CYP2C were determined by mass spectroscopy. Aortic ring and choroidal explant sprouting assays were used to investigate the effects of CYP2C inhibition and ω-3 LCPUFA derived CYP2C metabolic products on angiogenesis *ex vivo*. We found that inhibition of CYP2C activity by montelukast added to the protective effects of ω-3 LCPUFA on retinal and choroidal neovascularization by 30% and 20% respectively. In CYP2C8 over-expressing mice fed a ω-3 LCPUFA diet, montelukast suppressed retinal and choroidal neovascularization by 36% and 39% and reduced the plasma levels of CYP2C8 products. Soluble epoxide hydrolase inhibition, which blocks breakdown and inactivation of CYP2C ω-3 LCPUFA-derived active metabolites, increased OIR and CNV *in vivo*. Exposure to selected ω-3 LCPUFA metabolites of CYP2C significantly reversed

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Disclosures

None

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the suppression of both angiogenesis *ex vivo* and endothelial cell functions *in vitro* by the CYP2C inhibitor montelukast.

Conclusion—Inhibition of CYP2C activity adds to the protective effects of ω-3 LCPUFA on pathological retinal and choroidal neovascularization.

Keywords

CYP2C inhibitor; ω-3 LCPUFA; pathological angiogenesis

Introduction

Pathological ocular angiogenesis comprising retinopathy and choroidal neovascularization is a leading cause of vision loss in all age groups including retinopathy of prematurity (ROP) in children, diabetic retinopathy (DR) and age-related macular degeneration (AMD) in adults[1–3]. Pathological retinal and choroidal neovascularization can be suppressed temporarily with anti-angiogenic agents. In particular, anti-vascular endothelial growth factor (VEGF) molecules have been used successfully for the treatment of neovascular AMD, DR and ROP, but with some adverse effects[4, 5]. Suppressing VEGF signaling does not address the underlying causes of neovascularization. Frequent intraocular injections of anti-VEGF drugs also carry a cumulative risk of complications, including the potential of long-term suppression of the beneficial neurotrophic effects of VEGF on neural retina[6, 7]. Identification of additional therapies with fewer adverse effects is highly desirable.

Beyond their roles as energy substrates and structural constituents of membranes, essential dietary lipids and their metabolites also regulate retinal and choroidal neovascularization[8–10]. In particular, long-chain polyunsaturated fatty acids (LCPUFA) influence eye diseases[11]. The ω-3 LCPUFA, docosahexaenoic acid (DHA) and ω-6 LCPUFA, arachidonic acid (AA), are present in retinal neural and vascular cell membrane phospholipids[12]. DHA is present in the retina at a higher concentration (20% of lipids) than in any other tissue, and adequate dietary intake is associated with a reduced risk of ROP, DR and AMD[13–16]. Proliferative retinopathy and neovascular AMD are respectively modeled in the mouse eye by oxygen-induced retinopathy (OIR) and laser-induced choroidal neovascularization (CNV)[17, 18]. Dietary intake of ω-3 versus ω-6 LCPUFA reduces pathological angiogenesis in retina and choroid in these models[8, 18].

Both ω-3 and ω-6 LCPUFA are metabolized by at least three major pathways: cyclooxygenases (COXs), lipoxygenases (LOXs) and cytochrome P450 oxidases (CYPs). The metabolic products of ω-6 LCPUFA from these pathways are generally proangiogenic[11] whereas ω-3 LCPUFA metabolites of the first 2 pathways, such as COX-2 derived prostaglandin E3 and 5-LOX derived 4-hydroxy-docosahexaenoic acid, show anti-angiogenic effects[10, 19]. Our previous studies found that CYP2C products derived from ω-3 and ω-6 LCPUFA, particularly 19,20-epoxydocosapentaenoic acid (EDP) and 14,15-epoxyeicosatrienoic acid (EET), are pro-angiogenic and involved in CYP2C regulation of retinal neovascularization[20], partially counteracting the overall anti-angiogenic effects of ω-3 LCPUFA. These bioactive epoxides are further hydrolyzed by soluble epoxide hydrolase (sEH) into less active diols, such as 19,20-dihydroxy-docosapentaenoic acid

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(19,20-DiHDPA) and 14,15-dihydroxy-eicosatrienoic acid (14,15-DHET)[20, 21]. These findings suggest that inhibition of CYP2C activity might add to the protective effects of ω-3 LCPUFA on pathological retinal and choroidal neovascularization.

(R,E)-2-1-1-3-2-7-chloroquinolin-2-yl vinyl phenyl-3-2-2-hydroxypropan-2-yl phenyl propylthio methyl cyclopropyl acetic acid, also known as montelukast, was identified as a potent and selective CYP2C8 inhibitor (among human CYP2C enzymes) with a very high affinity (IC50 = 9.22 ± 0.88 nM) *in vitro*[22, 23] suggesting its potential as an effective antagonist of CYP2C-catalyzed metabolism[22, 23]. Montelukast suppresses colon cancer development by inhibiting tumor angiogenesis and vascular permeability[24, 25]. Montelukast was originally developed as a cysteinyl leukotriene receptor 1 (CysLTR1) antagonist, and is widely used for the treatment of asthma and seasonal allergies[26]. Whereas CYP2C enzymes are expressed in endothelial cells[20, 27], CysLTR1 is primarily expressed in leukocytes and macrophages, but not in endothelial cells[28, 29], suggesting the effects of montelukast on angiogenesis and endothelial cells may be mediated by inhibition of CYP2C activity unrelated to its role as a CysLTR1 antagonist.

This study showed that inhibition of CYP2C activity by montelukast decreased the plasma levels of CYP2C metabolites and added to the protective effects of ω-3 LCPUFA on pathological ocular neovascularization. In contrast to inhibition of CYP2C, increasing plasma levels of CYP2C products with a sEH inhibitor, that blocks the breakdown of CYP2C ω-3 LCPUFA bioactive epoxides products into less active diols[20, 30, 31] promoted ocular neovascularization *in vivo*. We also found that selected ω-3 LCPUFA metabolites of CYP2C reversed the inhibition of montelukast on angiogenesis *ex vivo* and endothelial cell functions *in vitro*.

Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

Results

CYP2C inhibition added to the protective effects of ω-3 LCPUFA on retinal and choroidal neovascularization

To test the hypothesis that inhibiting CYP2C activity adds to ω-3 LCPUFA inhibition of pathological retinal and choroidal neovascularization, we subjected C57BL/6 mice fed with either ω-6 or ω-3 LCPUFA enriched diets (as 2% of total dietary fatty acids (2% AA without any ω-3 LCPUFA or 1% DHA and 1% EPA with any ω-6 LCPUFA) to either OIR or laser-induced CNV, and treated them with either the CYP2C inhibitor montelukast or vehicle control. At postnatal day (P) 17, OIR pups fed a ω-3 (versus ω-6) LCPUFA enriched diet had 19% ($P = 0.046$) less retinal neovascularization, which was further reduced by the inhibition of CYP2C by 30% ($P = 4.4\times10^{-7}$) (Figure 1A&B). Moreover, adult mice fed a ω-3 (versus ω-6) LCPUFA enriched diet had 10% ($P = 0.020$) reduction of CNV lesion area at 7 days after laser photocoagulation, and inhibition of CYP2C further reduced the CNV lesion area by 20% ($P = 6.6\times10^{-6}$) (Figure 1C&D). Although the effects of CYP2C inhibition are not specific for, or require ω-3 LCPUFA, these results suggested that the

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CYP2C inhibitor montelukast enhances the overall protective effects of ω-3 LCPUFA on both retinal and choroidal neovascularization.

Inhibition of CYP2C8 activity suppressed retinal and choroidal neovascularization and CYP2C8 products

To further examine the potential for CYP2C inhibition to augment the protective effect of ω-3 LCPUFA on ocular neovascularization, we treated Tie2-driven human *CYP2C8* overexpressing mice and their wild-type littermates on a ω-3 LCPUFA enriched diet with montelukast or vehicle control daily in the OIR and laser-induced CNV models. CYP2C8 overexpression increased retinal neovascularization in OIR by 34% ($P = 3.0\times10^{-5}$), which was suppressed by 36% ($P = 1.7\times10^{-5}$) at P17 with montelukast treatment (Figure 2A & Supplemental Figure IA). CYP2C8 transgenic mice fed a ω-3 LCPUFA enriched diet had 25% ($P = 3.4\times10^{-7}$) more choroidal neovascularization than wild-type littermates, which was suppressed by 39% ($P = 3.4\times10^{-16}$) with montelukast treatment (Figure 2B & Supplemental Figure IB). We chose to examine the plasma levels of AA and DHA metabolites of CYP2C, 14,15-EET and 19,20-EDP, and their metabolic products of sEH (Figure 2C) because our previous studies revealed their pro-angiogenic effects on pathological retinal and choroidal neovascularization. The decreased pathological angiogenesis with CYP2C inhibition was accompanied by 24% ($P = 0.011$) lower plasma levels of the bioactive CYP2C8 product derived from ω-3 LCPUFA, 19,20-EDP (Figure 2D). The production of other LCPUFA metabolites of CYP2C8 was also suppressed by montelukast (Supplemental Table I). Also, montelukast reversed the induction of retinal (OIR) and choroidal neovascularization (CNV) in the Tie2-driven *CYP2C8* transgenic mice on a ω-6 LCPUFA enriched diet by 51% ($P = 8.6\times10^{-4}$) and 47% ($P = 6.5\times10^{-7}$) respectively (Supplemental Figure I&II), which was accompanied by 23% ($P = 0.031$) lower plasma levels of CYP2C8 products derived from ω-6 LCPUFA, such as 14,15-EET, without changing CYP2C8 transcriptional levels (Supplemental Figure II & Table II). These results suggested that inhibition of CYP2C activity by montelukast is associated with suppression of pathological angiogenesis in OIR and laser-induced CNV.

sEH inhibition increased retinal and choroidal neovascularization *in vivo* and angiogenesis *ex vivo*

To investigate the regulatory effects of the CYP2C/sEH pathway and the metabolites on pathological retinal and choroidal neovascularization, we treated C57BL/6J mice with the sEH inhibitor in the piperidine series 1770 (1-trifluoromethoxyphenyl1-3-(1-propionylpiperidin-4-yl)urea)[32, 33] or vehicle control in both OIR and laser-induced CNV models. We found sEH inhibition increased ocular neovascularization by 27% ($P = 3.3\times10^{-8}$) and 26% ($P = 9.7\times10^{-6}$) respectively in OIR and laser-induced CNV (Figure 3A&B & Supplemental Figure IIIA&B), which was also accompanied by 56±17% ($P = 0.016$) and 105% ($P = 0.032$) higher plasma levels of the bioactive epoxides 19,20-EDP and 14,15-EET (Figure 3C&D). However, neither sEH nor CYP2C inhibitor affected vaso-obliteration in OIR (Supplemental Figure IIIC&D). The sEH inhibitor 1770 significantly decreased the plasma levels of diols but had no effect on LCPUFA metabolites through other pathways (Supplemental Table III). The ratios of both 19,20-EDP:DiHDPA and 14,15-EET:DHET were increased by 153% ($P = 0.035$) and 44% ($P = 0.020$) respectively by

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addition of the sEH inhibitor 1770 (Supplemental Figure IVA&B). The transcriptional levels of neither CYP2C nor sEH were significantly altered by sEH inhibition (Supplemental Figure IVC&D). Moreover, sEH inhibition yielded 48% ($P = 5.0\times10^{-5}$) and 42% ($P = 0.046$) increases in sprouting of both aortic rings and choroidal explants (Figure 4). These results indicated the association of ω-3 LCPUFA metabolites of CYP2C with neovascularization.

19,20-EDP reversed the inhibition of angiogenesis *ex vivo* by CYP2C inhibition

To further investigate if inhibition of CYP2C and lower levels of CYP2C products derived from ω-3 LCPUFA suppressed angiogenesis, we examined the effects of the CYP2C inhibitor montelukast in the presence of DHA, which would provide metabolites from many pathways including beneficial products or one of its CYP2C metabolites, 19,20-EDP, on tissue explants. In the aortic ring sprouting assay, there was 19% ($P = 0.030$) less sprouting area with DHA compared with control at day 6 (Supplemental Figure VA&B). Addition of montelukast further suppressed sprouting by 46% ($P = 0.0037$), which was consistent with results from the animal models *in vivo* suggesting that CYP2C products were pro-angiogenic. In the choroid sprouting assay, montelukast not only decreased the sprouting area by 29% ($P = 0.0087$), but also further increased the inhibitory effects of DHA by 28% ($P = 0.013$) (Supplemental Figure VC&D). In contrast, one of the DHA metabolites of CYP2C, 19,20-EDP, reversed the suppression of sprouting by montelukast in aortic rings and choroidal explants by 58% ($P = 4.3\times10^{-4}$) and 59% ($P = 4.2\times10^{-4}$) respectively (Figure 5). These data suggested that inhibition of CYP2C by montelukast suppressed angiogenesis *ex vivo*.

19,20-EDP reversed the inhibition of endothelial cell tubule formation *in vitro* by CYP2C inhibition

We examined the anti-angiogenic effects of the CYP2C inhibitor montelukast on endothelial cell tubule formation *in vitro* using human retinal microvascular endothelial cells (HRMECs). Cells treated with montelukast showed a 32% reduction ($P = 3.5\times10^{-4}$) in tubule formation compared with cells treated with control (Figure 6A&B). Addition of DHA further inhibited HRMEC tubule formation by 93% ($P = 0.0025$) without any effect on CYP2C8 expression (Supplemental Figure VI). However, 19,20-EDP reversed the suppression of HRMEC tubule formation by montelukast by 43% ($P = 7.4\times10^{-4}$) (Figure 6A&B). Neither montelukast nor 19,20-EDP had an effect on the transcription of CYP2C8 in HRMECs (Figure 6C). To further distinguish between CYP2C8 and CysLTR1 inhibitory effects of montelukast, we treated HRMECs with specific CYP2C8 or CysLTR1 siRNAs. CYP2C8, but not CysLTR1, knockdown inhibited HRMEC tubule formation by 64% (Supplemental Figure VIIA&B). Moreover, CysLTR1 expression levels were undetectable in HRMECs (Supplemental Figure VIIC). These data suggested that the lower levels of bioactive CYP2C products derived from ω-3 LCPUFA are involved in the impairment of endothelial cell functions by CYP2C inhibition.

Discussion

Increased dietary intake of ω-3 LCPUFA reduces pathological retinal and choroidal neovascularization[8]. While many ω-3 LCPUFA metabolites biosynthesized through COX

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and LOX pathways have anti-angiogenic effects, ω-3 LCPUFA metabolites of CYP2C promote both retinal neovascularization (OIR) and choroidal neovascularization (laser-induced CNV)[10, 19, 20]. In this study, we showed that inhibition of CYP2C activity by montelukast added to the protective effects of ω-3 LCPUFA on ocular neovascularization in the animal models of both OIR and laser-induced CNV. The increased neovascularization observed in transgenic mice overexpressing human CYP2C8 on a ω-3 LCPUFA enriched diet was substantially reversed with CYP2C inhibition, associated with lower plasma levels of CYP2C8 products derived from ω-3 LCPUFA without affecting CYP2C8 transcriptional levels. Montelukast treatment reduced both CNV and OIR in *CYP2C8* transgenic mice to the level of or below that of untreated wild-type littermates. In addition, inhibition of sEH significantly increased ocular neovascularization *in vivo* in association with increased plasma levels of the bioactive ω-3 LCPUFA metabolites of CYP2C. Addition of ω-3 LCPUFA metabolites of CYP2C reversed the suppression of angiogenesis *ex vivo* and endothelial cell functions *in vitro* by CYP2C inhibition.

CYP2C8 is present at high levels in human tissues and is involved in endothelial cell functions[27, 34]. In an *in vitro* screening of 209 frequently prescribed drugs to examine their potential to inhibit CYP2C8, montelukast was identified as a potent suppressor[22] and a selective inhibitor of CYP2C8 activity among all tested human CYP2C isoforms[23]. Mouse Cyp2C55 shares a high homology with human CYP2C8[35]. In our studies, montelukast reduced retinal and choroidal neovascularization not only in Tie2-driven *CYP2C8* (human) transgenic but also in wild-type mice, which suggests that montelukast inhibits not only exogenous human CYP2C8 but also endogenous mouse Cyp2C activity, such as Cyp2C55.

Montelukast was originally formulated as a specific antagonist for CysLTR1 and is broadly used to treat chronic asthma[26]. CysLTR1 is expressed in lung, spleen, leukocytes, macrophages and smooth muscle cells, and its activation leads to contraction and proliferation of smooth muscle, edema, and eosinophic migration[29, 36]. Studies of the effect of montelukast on angiogenesis are limited. Montelukast suppresses colon cancer growth through the inhibition of angiogenesis and inhibits angiogenesis *ex vivo* in rat thoracic aortic rings[24]. Our data show that montelukast inhibited angiogenesis *ex vivo* in mouse aortic rings and choroid explants. However, there is some controversy about the effects of montelukast on endothelial cells. Montelukast was reported to reduce vascular permeability by reducing VEGF expression[25], whereas others found that montelukast increases inter-cellular adhesion molecule 1 expression in human primary endothelial cells[37]. CysLTR1 is generally reported as absent in primary endothelial cells[28, 29], although one group reported its expression in a human endothelial cell line and that montelukast inhibited endothelial cell migration by inhibiting the extracellular signal-regulated kinase pathway[38]. We also observed the inhibition of human retinal endothelial cell migration and VEGF-induced extracellular signal-regulated kinase activation by montelukast (Supplemental Figure VIII), but failed to detect the expression of CysLTR1 in human primary retinal endothelial cells (Supplemental VIIC). Our results suggested that montelukast impairs endothelial cell functions by inhibiting CYP2C activity. The CYP2C inhibitor montelukast is approved by the United States Food and Drug Administration and has been used to treat asthma for decades so could be repurposed to treat neovascular eye diseases. Our findings enhance our knowledge of tissue-specific effects of montelukast and its mechanism of angiogenesis regulation.

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Compared with ω-6 LCPUFA, ω-3 LCPUFA reduce pathological retinal and choroidal neovascularization[8, 18]. The ω-3 LCPUFA DHA inhibits angiogenesis *ex vivo* and endothelial functions *in vitro*, which is consistent with previous reports and likely related to its metabolites produced through COX and LOX, rather than CYP pathways[9, 10, 20]. DHA does not appreciably alter the effects of CYP2C inhibition, and further increases the inhibition of angiogenesis, suggesting that DHA has parallel effects in the co-treatment with a CYP2C inhibitor. In contrast to the modest effects of DHA on the inhibition of montelukast, addition of CYP2C products derived from DHA, such as 19,20-EDP, reversed the inhibitory effects of this CYP2C inhibitor on angiogenesis *ex vivo* and endothelial cell functions *in vitro*, suggesting that montelukast functions upstream of CYP2C products. Moreover, increased plasma levels of CYP2C products derived from ω-3 LCPUFA with inhibition of sEH activity also promoted neovascularization, which was accompanied by increased levels of ω-3 LCPUFA metabolites of CYP2C in plasma. Our results suggested that inhibition of CYP2C activity suppresses endothelial cell growth during angiogenesis by lowering the levels of CYP2C products. More clinical research on biological effects of CYP2C inhibition on neovascularization will expand our understanding about the mechanism of CYP2C/sEH pathway regulation of angiogenesis.

CYP2C inhibition reversed the induction of retinal and choroidal neovascularization in CYP2C8 overexpressing mice fed with either a ω-3 or ω-6 LCPUFA enriched diet. These results suggest that CYP2C products derived from both ω-3 and ω-6 LCPUFA are pro-angiogenic in the retina and choroid. A previous study showed that 19,20-EDP inhibited tumor growth and human umbilical vein endothelial cell functions in vitro by suppressing VEGF-C, but not VEGF-A[39]. In our studies, no change in VEGF-C expression was observed in either Tie2-driven CYP2C8 transgenic or CYP2C inhibitor-treated retina (Supplemental Figure IX). The different expression pattern of VEGF-A and VEGF-C in the retina and in tumor might contribute to the different effects of the CYP2C metabolite observed, indicating a tissue-specific role of 19,20-EDP. Despite much research on the potent effects of other CYP2C metabolites, such as EDPs and EETs, in many physiological and pathological processes, knowledge about the molecular mechanism or regulation of angiogenesis and endothelial cell behaviors is still limited. A recent study indicated that 11,12-EET promotes hematopoietic stem and progenitor cell specification by increasing activator protein 1 and runx1 transcription through phosphatidylinositol-3-OH kinase pathway[40], which has also been implicated in advanced AMD by our previous work[41]. More research on the direct target and downstream pathways of CYP2C metabolites is needed.

Our inferences were strengthened on the basis of 5 additional lines of evidence: 1) intravitreal injections of trimethoprim, an antibiotic known to inhibit CYP2C8, is effective in treatment of human toxoplasma retinochorioditis, a disease affecting the retinal vasculature[42, 43]; 2) injections of cationic liposome-encapsulated paclitaxel, a substrate of CYP2C8, reduces the volume of choroidal neovascularization in an animal model of pathologic choroidal angiogenesis[44]; 3) exposure to thiazolidinedione, an anti-diabetic agent known to inhibit CYP2C8, significantly reduces the extent of retinal neovascular pathology in OIR mice[45] – effects in this report were attributed to a pioglitazone-induced increase in adiponectin, a ω-3 LCPUFA-modulated protective factor for pathological retinal and choroidal neovascularization[46]; 4) CYP2C8-inhibiting thiazolidinediones also act as trans-

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activators of the peroxisome proliferator activated receptor gamma transcription response element, which is part of an ω-3 LCPUFA-sensing signaling system implicated in ROP, DR and AMD[9, 47, 48]; and 5) pull-down and co-immunopreciptation studies confirmed the binding of CYP2C8 with alpha 2-macroglobulin, a major protease inhibitor that acts on and is cleaved by matrix metallopeptidase 9 an AMD-associated collagenase essential for clearing space for sprouting vessels within the angiogenic cascade[49, 50].

In summary, our study found that inhibition of CYP2C inhibited pathological retinal and choroidal neovascularization by lowering the levels of CYP2C products from both ω-6 and ω-3 LCPUFA. ω-3 LCPUFA may help prevent retinal and choroidal neovascularization. Our findings suggest enhanced protective effects of ω-3 LCPUFA against pathological angiogenesis with CYP2C inhibition. Montelukast is a potential therapeutic to treat neovascular eye diseases. Dietary ω-3 LCPUFA DHA supplementation with CYP2C inhibition is likely to benefit retinal and choroidal neovascularization.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AA	arachidonic acid
AMD	age-related macular degeneration
CNV	choroidal neovascularization
COX	cyclooxygenase
CYP	cytochrome P450 oxidase
CysLTR	cysteinyl leukotriene receptor
DHA	docosahexaenoic acid
DHET	dihydroxy-eicosatrienoic acid
DiHDPA	dihydroxy-docosapentaenoic acid

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DR	diabetic retinopathy
EDP	epoxydocosapentaenoic acid
EET	epoxyeicosatrienoic acid
HRMEC	human retinal microvascular endothelial cell
LCPUFA	long-chain polyunsaturated fatty acids
LOX	lipoxygenase
OIR	oxygen-induced retinopathy
ROP	retinopathy of prematurity
sEH	soluble epoxide hydrolase
VEGF	vascular endothelial growth factor

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Highlights

- Cytochrome P450 oxidase 2C inhibitor reduces retinal and choroidal neovascularization.
- Soluble epoxide hydrolase inhibitor induces retinal and choroidal neovascularization.
- Cytochrome P450 oxidase 2C inhibitor adds to the protective effects of ω-3 long-chain polyunsaturated fatty acids on pathological retinal and choroidal neovascularization.
- cytochrome P450 oxidase Inhibitor presents a new therapeutic approach for prevention of neovascular eye diseases.
- Montelukast as an approved pharmaceutical on the market could be repurposed for treating neovascular eye diseases.

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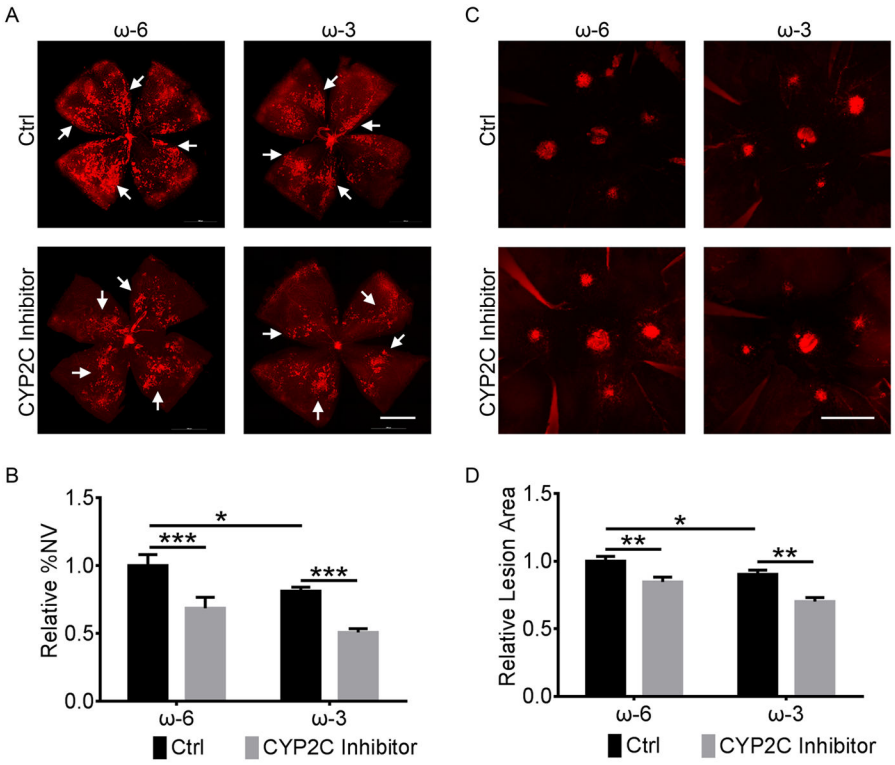

Figure 1. CYP2C inhibitor added to the protective effects of ω-3 LCPUFA on retinal and choroidal neovascularization

Representative images of retinal (A) and choroidal (C) flat-mounts from OIR mice fed with a ω-6 or ω-3 LCPUFA enriched diet and intraperitoneally injected with the CYP2C inhibitor montelukast (1 mg/kg) or 10% DMSO control daily from postnatal day (P) 12 to P16 for OIR or from day 0 to day 6 after laser photocoagulation for CNV. Scale bar, 1 mm (A), 500 μm (C). CYP2C inhibitor augmented the suppression of retinal (B) and choroidal (D) neovascularization (NV) by ω-3 LCPUFA. n = 11 mice/group. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

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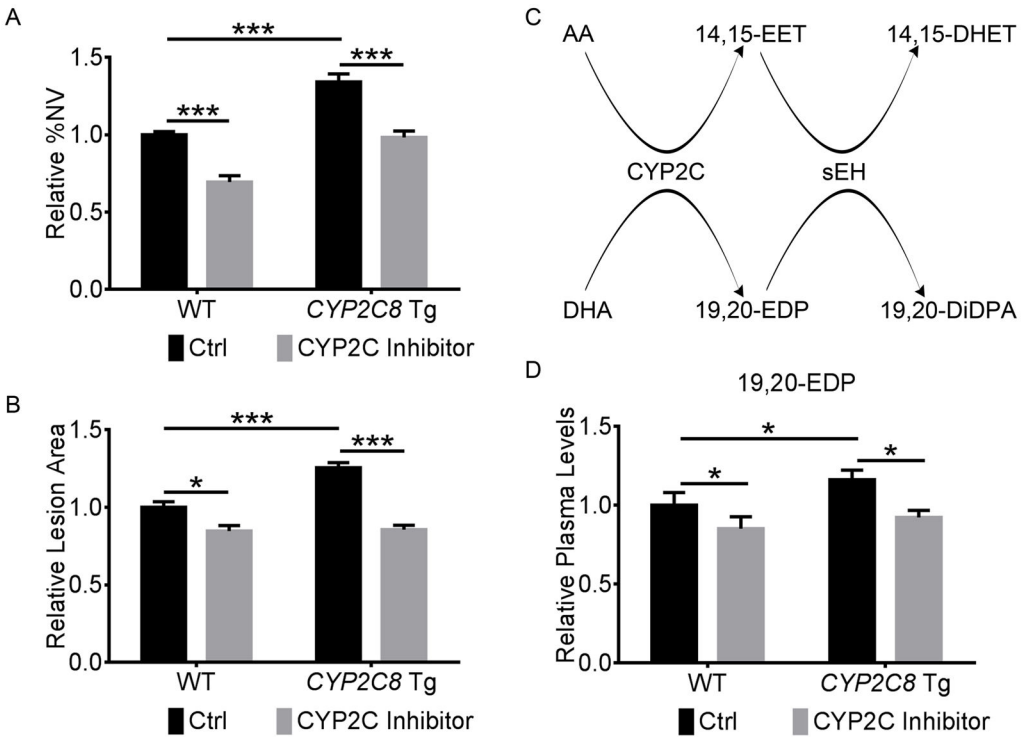

Figure 2. CYP2C inhibitor reduced retinal and choroidal neovascularization and CYP2C8 products

CYP2C inhibitor reversed the induction of retinal (A) and choroidal (B) neovascularization (NV) by *CYP2C8* overexpression in the mouse OIR and laser-induced CNV models. Tie2-driven *CYP2C8* transgenic (Tg) mice and wild-type (WT) littermate controls fed with a ω-3 LCPUFA enriched diet were intraperitoneally injected with the CYP2C inhibitor montelukast (1 mg/kg) or 10% DMSO control daily from P12 to P16 for OIR or from day 0 to day 6 after laser photocoagulation for CNV. C, Schematic diagram of CYP2C8 and sEH metabolism of DHA and AA. D, CYP2C inhibitor reversed the induction of plasma levels of DHA metabolites downstream of CYP2C8, 19,20-EDP, by *CYP2C8* overexpression in mice. n = 10–16 mice/group. **P* < 0.05; *** P < 0.001.

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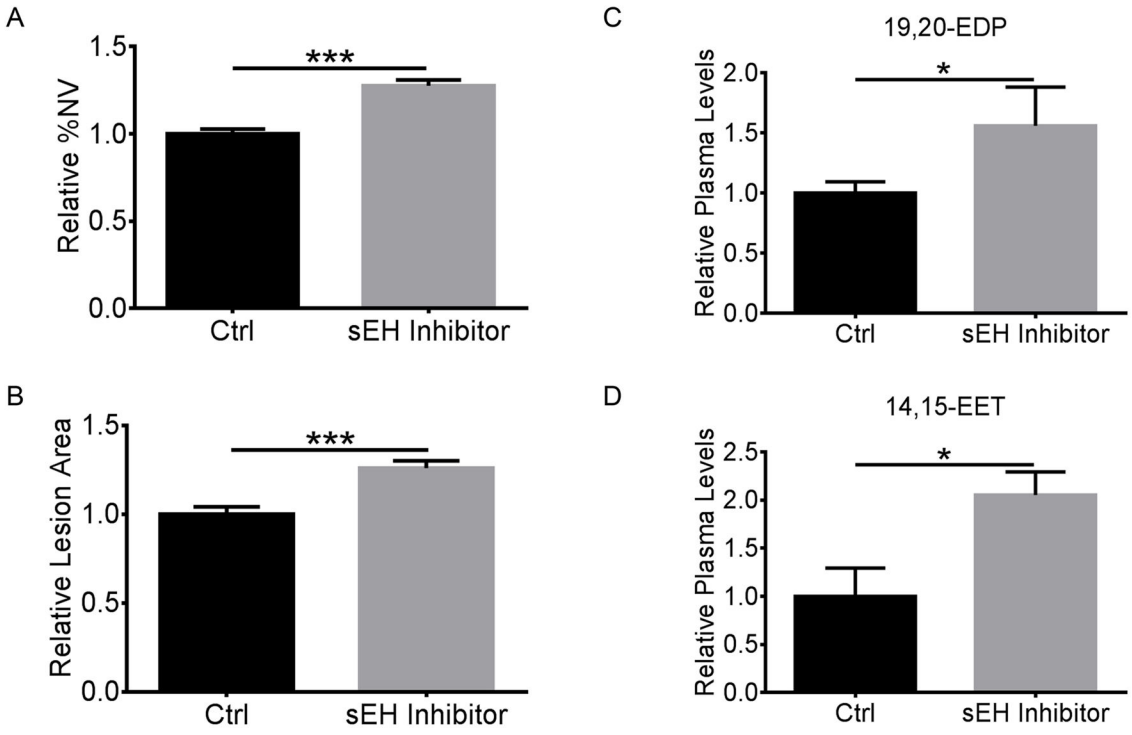

Figure 3. sEH inhibitor aggravated retinal and choroidal neovascularization

sEH inhibitor increased P17 retinal (A) and choroidal (B) neovascularization (NV) in OIR and laser-induced CNV mice. C57BL/6J mice were intraperitoneally injected with the sEH inhibitor 1770 (0.3 mg/kg) and 10% DMSO control daily from P12 to P16 for OIR (n = 26–27 mice/group) or from day 0 to day 6 after laser photocoagulation for CNV (n = 11 mice/group). sEH inhibitor increased plasma levels of 19,20-EDP (C) and 14,15-EET (D) in mice. n = 4 mice/group. * $P < 0.05$; *** $P < 0.001$.

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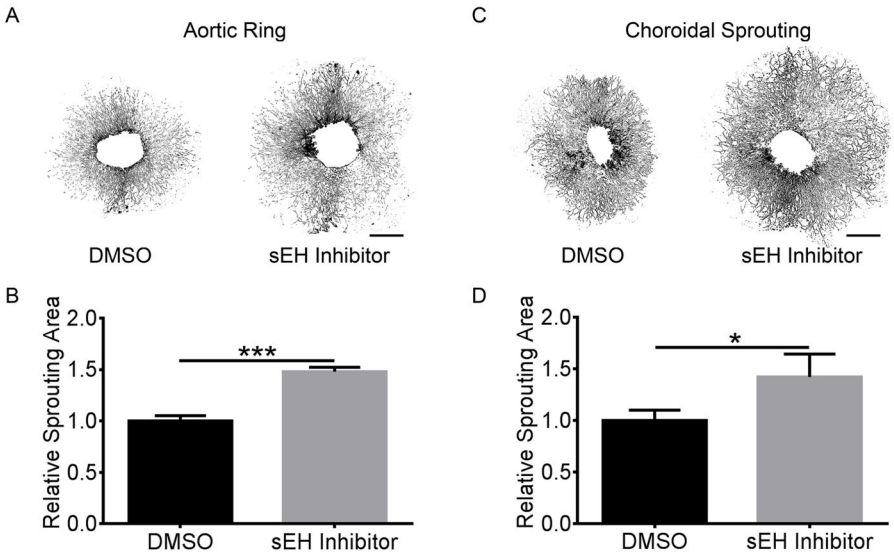

Figure 4. sEH inhibitor promoted angiogenesis *ex vivo*

Representative images of aortic rings (A) and choroidal (C) sprouting treated with the sEH inhibitor 1770 (20 μg/ml) or 0.2% DMSO as control for 6 days after tissue planting. Scale bar, 1 mm. sEH inhibitor promoted aortic ring (B) and choroidal (D) sprouting. n = 5. * $P < 0.05$; *** $P < 0.001$.

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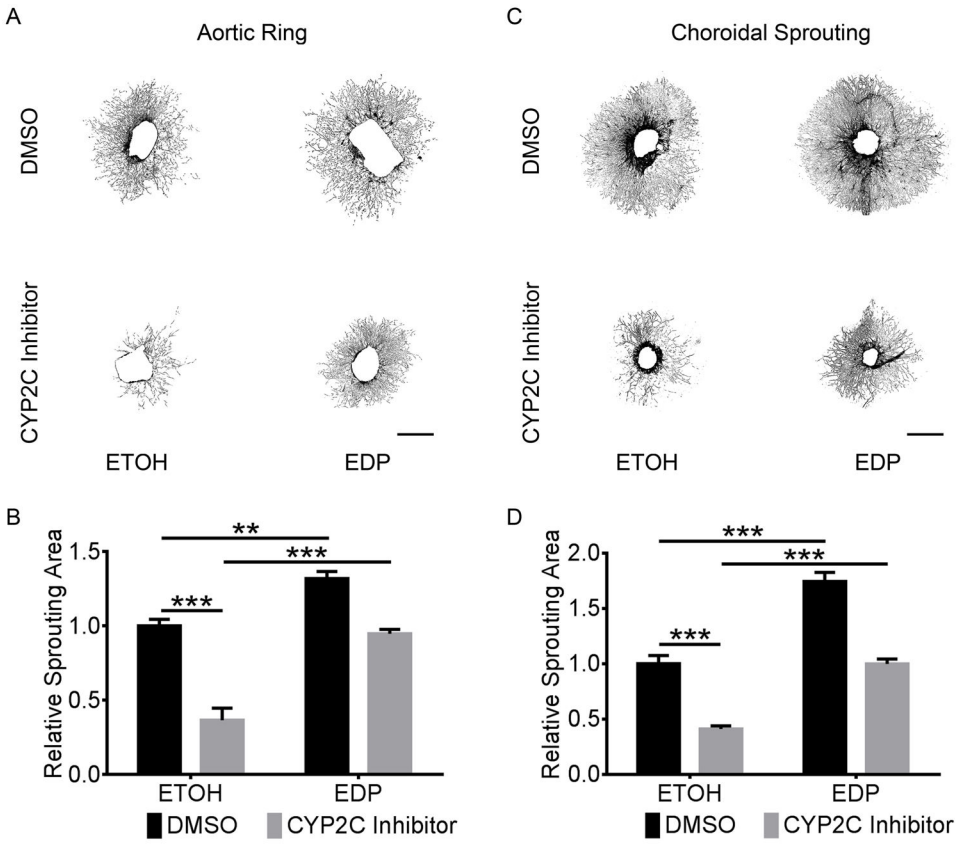

Figure 5. 19,20-EDP reversed the inhibition of angiogenesis *ex vivo* by CYP2C inhibition

Representative images of aortic rings (A) and choroidal (C) treated with the CYP2C inhibitor montelukast (20 μg/ml) or 0.2% DMSO control, and 19,20-EDP (1 μM) or ethanol (ETOH) vehicle control for 6 days after tissue planting. Scale bar, 1 mm. 19,20-EDP rescued the inhibition of aortic ring (B) and choroidal (D) sprouting by CYP2C inhibition. n = 5. ** $P < 0.01$; *** $P < 0.001$.

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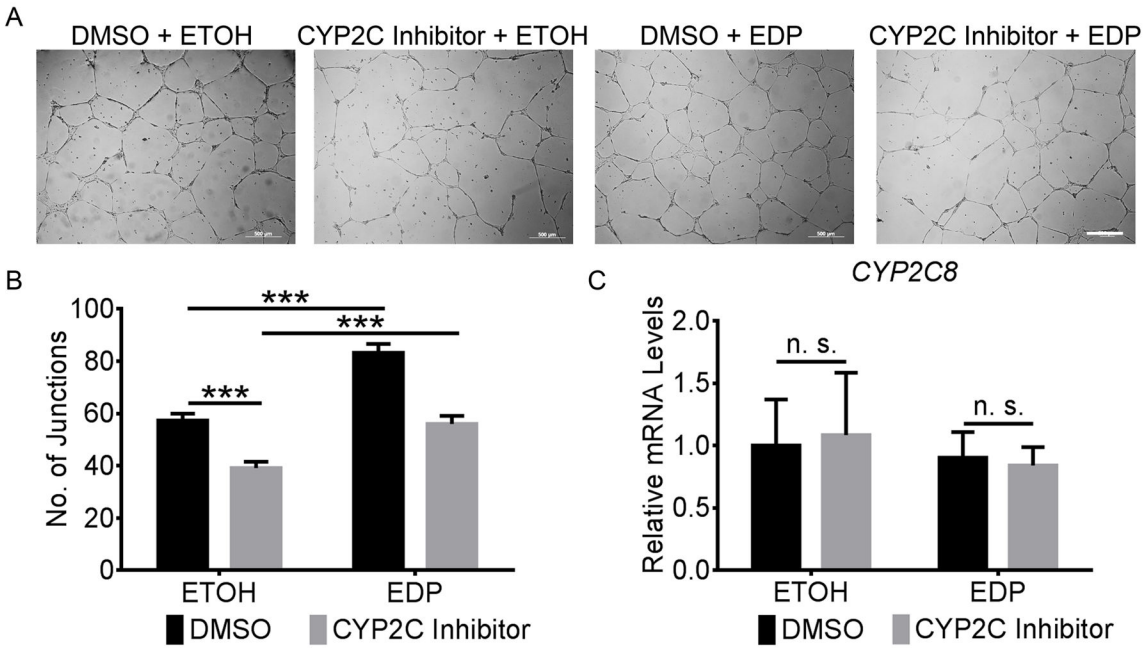

Figure 6. 19,20-EDP reversed the inhibition of human retinal endothelial cell (HRMEC) tubule formation by CYP2C inhibition

A, Representative photos of HRMECs grown on Matrigel for 6 hour under the conditions of the CYP2C inhibitor montelukast (20 μg/ml) or 0.2% DMSO control, and 19,20-EDP (1 μM) or ETOH vehicle control. Scale bar, 500 μm. B, 19,20-EDP rescued the inhibition of endothelial cell tubule formation by CYP2C inhibition. C, Neither CYP2C inhibitor nor 19,20-EDP had effects on CYP2C8 transcriptional levels in HRMECs. n = 8. *** $P < 0.001$; n. s., not significant.