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Review Article

Epoxyeicosatrienoic acids and soluble epoxide hydrolase: potential therapeutic targets for inflammation and its induced carcinogenesis

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Abstract: Chronic inflammation is an important factor contributing to human carcinoma, and non-steroidal anti-inflammatory drugs (NSAIDs) have been shown to have a preventive effect in the development of various types of carcinoma. However, NSAIDs also have adverse side effects including increased cardiovascular events, making them less than ideal for routine chemoprevention. Soluble epoxide hydrolase (sEH) is an enzyme that converts endogenous anti-inflammatory compounds, the epoxyeicosatrienoic acids (EETs), to the less anti-inflammatory dihydroxyeicosatrienoic acids (DHETs). Inhibition of sEH, by a highly selective and potent sEH inhibitor (sEHI), increases EETs leading to decreased inflammation. In our studies, administration of a sEHI in mouse colitis models led to decreased ulcer incidence and number of ulcers compared to controls, with no adverse side effects seen. In human tissue, sEH showed an increase in expression, as seen immunohistochemically, in ulcerative colitis (UC), UC-induced dysplasia, and UC-induced carcinoma. Thus, inhibition of sEH may be a novel biomarker and potential therapeutic target in inflammation and inflammation-induced carcinoma.

Keywords: Epoxyeicosatrienoic acids (EETs), soluble epoxide hydrolase (sEH), dihydroxyeicosatrienoic acids (DHETs), inflammation, non-steroidal anti-inflammatory drugs (NSAIDs), therapy, carcinogenesis, cancer

Introduction

The epoxyeicosatrienoic acids (EETs), products of arachidonic acid metabolism via the cytochrome P450 enzyme-mediated pathway, are key anti-inflammatory mediators. Soluble epoxide hydrolase (sEH) hydrolyzes EETs to dihydroxyeicosatrienoic acids (DHETs), which have dramatically reduced biological activity [1]. sEH inhibitors (sEHIs) are compounds that inhibit sEH activity and result in a variety of beneficial cardiovascular effects and anti-inflammatory effects with no toxicities seen in animal models to date. sEHIs have been shown to affect peroxisome proliferator-activated receptor activity, nuclear factor-kB (NF-kB), and key enzymes in arachidonic acid metabolism, including cyclooxygenase (COX) and lipoxygenase (LOX). Although many studies have illustrated a decreased risk of developing colorectal adenomas and carcinoma with the use of non-steroidal anti-inflammatory drugs (NSAIDs) and cyclooxygenase-2 (COX-2) inhibitors, the risk of adverse cardiovascular events is a major concern. Our hypothesis is that sEH may potentially show the same benefits as NSAIDs and COX-2 inhibitors without the toxicities. In this review, we focus on linking the EET-sEH pathway to inflammation and carcinogenesis from experimental evidence to potential therapeutic application.

Soluble epoxide hydrolase (sEH)

Epoxide hydrolases are a family of enzymes that add water to the epoxide group of compounds to form the corresponding diols (**Figure 1**). The epoxide hydrolase family is divided into six main subtypes: soluble epoxide hydrolase (sEH), microsomal epoxide hydrolase (mEH), leukotriene A_4 hydrolase, hepoxilin A_3 hydrolase, and cholesterol 5,6-oxide hydrolase [2]. sEH (also known as cytosolic EH) is responsible for metabolizing

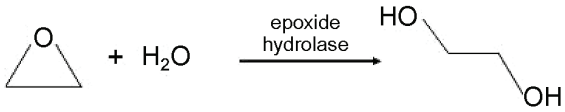

Figure 1. Epoxide hydrolase catalyzes diol formation by adding a water group to an epoxide.

EETs to DHETs. The human sEH is encoded by the EPHX-2 gene [3]. EPHX-2 is located on the short arm of chromosome 8 (8p21-p12), is nearly 45kb in length and consists of 19 exons [4]. The sEH in mammals is a homodimer composed of two 60-kDa monomers. Each monomer has an N-terminal domain with lipid phosphatase activity and uncertain physiologic role [5,6], and a carboxy-terminal domain composed of an α/β hydrolase fold with epoxide hydrolase activity [7]. The amino-terminal domain and carboxy-terminal domain are functionally independent of one another, and inhibition of one domain has no effect on the enzymatic activity of the other domain [5].

sEH is distributed in various tissues with high levels of sEH in intestine, liver, kidney, brain and vasculature and lower levels of sEH in lung, spleen and testes [8-11]. Intracellularly, sEH is located mainly in the cytosol, but is also located in peroxisomes [12-14]. In human hepatocytes and renal proximal tubules, sEH localizes to both the cytosol and peroxisomes, but is exclusively cytosolic in other sEH containing cells such as pancreatic islet cells, intestinal epithelium, anterior pituitary cells, adrenal gland, endometrium, lymphoid follicles, prostate ductal epithelium, and blood vessels [15]. Functional significance of different subcellular locations of sEH is not known.

Cytochrome P450 epoxygenases mediate active eicosanoids

Arachidonic acid (AA) is metabolized by the Cyclooxygenase (COX), Lipoxygenase (LOX), and cytochrome P450 (CYP 450) epoxygenase pathways to generate biologically active mediators including prostaglandins, leukotrienes and eicosanoids that are involved in various biologic events as well as several disease processes including inflammation, as shown in **Figure 2**. The cytochrome P450 epoxygenase pathway leads to the formation of 19- and 20- hydroxyeicosatetraenoic acid (19- and 20- HETE) and the epoxyeicosatrienoic acids (EETs) [16,17]. The CYP2C and CYP2J enzymes convert arachidonic acid to four EET regioisomers, -EET [5,6], -EET [8,9], -EET [11,12], and –EET [14,15]. The epoxide group can attach to either side of the double bond in arachidonic acid, resulting in two differ-

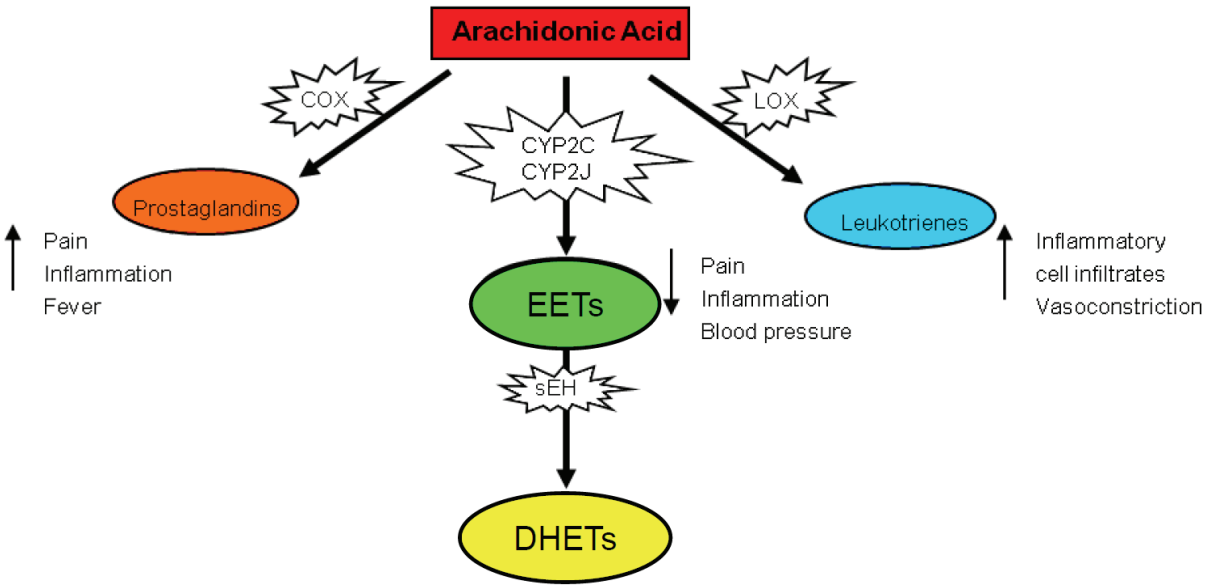

Figure 2. Arachidonic acid metabolism and the physiologic effects of arachidonic acid metabolites. COX = cyclooxygenase, CYP2C = cytochrome p450 2C, CYP2J = cytochrome p450 2J, DHETs = dihydroxyeicosatrienoic acids, EETs = epoxyeicosatrienoic acids, LOX = lipoxygenase, sEH = soluble epoxide hydrolase.

ent R/S and S/R enantiomers for each EET regioisomer[16]. The CYP 450 epoxygenases are capable of forming all four EET regioisomers, but -EET [11,12], and -EET [14,15] predominate [16]. EETs have a number of physiological functions including angiogenesis, apoptosis, fibrinolysis, mitogenesis, hormone secretion, vasodilation, bronchodilation and anti-inflammatory effects.

The EETs act in both an autocrine and paracrine manner and are located in various organs including the liver, kidney, and heart [18-20]. All EETs are incorporated into cellular phospholipids via a coenzyme A-dependent manner [18,21]. One possible mechanism for EET release is activation of a cell containing a CYP 450 epoxygenase. Upon activation, arachidonic acid is released from the phospholipids and the CYP 450 epoxygenase then synthesizes the EET [22]. Another possible mechanism is that the EETs are already preformed and stored in phospholipids and when a cell is "activated", the phospholipase releases the preformed EETs [23].

Once EETs are formed, it is thought that they can exert their autocrine and paracrine effects through two possible mechanisms. The first postulated mechanism is that EETs bind to a membrane receptor, which then activates an intracellular cascade affecting ion channels or DNA expression. Several studies support the idea of a G protein-coupled receptor [24-26] , while other studies propose tyrosine kinase and mitogen-activated protein kinase (MAPK) pathways [27-30]. The second postulated mechanism is through direct interaction of EETs with signal transduction pathways, transcription factors or ion channels.

EETs are metabolized through several different pathways including β-oxidation[31], chain elongation [9,32,33], hydration by soluble epoxide hydrolase (sEH), and to a lesser extent, EETs can be metabolized by CYP ω-oxidases [34], cyclooxygenase (COX) [35, 36], lipoxygenase (LOX) [37] and glutathione-S-transferase (GSH) [38]. The main catabolic pathway of EETs is the conversion to DHETs by sEH. EETs have increased anti-inflammatory properties compared to DHETs [1], but EETs are turned over so rapidly it is difficult to demonstrate this *in vivo*. However, the addition of a sEH inhibitor is an efficient way to increase EETs function physiologically.

EETs-sEH pathway and its anti-inflammation activity

The role of the EET-sEH pathway in inflammation has been assessed in rodent models [1, 39 -44]. The initial findings of the anti-inflammatory properties of EETs described by Node et al demonstrated that physiologic concentrations of EETs or overexpression of CYP2J2 (the CYP450 enzyme that transforms AA to EETs) inhibits inflammation through decreasing cytokine-induced endothelial cell adhesion molecule expression, and preventing leukocyte adhesion to vascular walls and tissues by a mechanism involving inhibition of (NF-kB) [45]. Furthermore, when EETs are stabilized by sEH inhibitors, they have dramatically increased anti-inflammatory properties [1, 39-44, 46]. Using rodent models, investigators have demonstrated that sEH inhibitors are effective in the treatment of inflammatory diseases [42]. Mice with lipopolysaccharide(LPS)-induced systemic inflammation, treated with the sEH inhibitor 12-(3-adamantane-1-yl-ureido)-dodecanoic acid n-butyl ester (AUDA-nBE), showed reduced production of nitric oxide, cytokines, and pro-inflammatory lipid mediators and significantly improved survival [42, 47]. In another study, daily subcutaneous injections of the sEH inhibitor AUDA-nBE were administered to spontaneously hypertensive rats that were either exposed to filtered air or tobacco smoke for a period of 3 days (6 h/day). The tobacco smoke-exposed rats treated with AUDA-nBE showed a significant decrease in bronchoalveolar inflammatory cells, including significant reductions in neutrophils, alveolar macrophages, and lymphocytes [43]; and a combination of sEH inhibitor and exogenously administered EETs is even more significant in reducing tobacco smoke-induced inflammation [43].

EETs also increase peroxisome proliferator-activated receptor-gamma (PPAR-γ) transcription activity, which is a key molecular event involved in inhibiting NF-κB. Evidence from cell culture models shows that blocking PPARγ activity reduces the EET/sEH inhibitor-mediated anti-inflammatory effect, indicating PPARγ is an effector of EETs [48]. PPARγ exerts an anti-inflammatory effect by interfering with the activity of inflammatory transcription factors, such as Activator Protein-1 (AP-1), Signal Transducers

and Activators of Transcription (STAT), and nuclear factor κB (NF-κB) [49, 50]. Therefore, activation of PPARγ could be a key pathway to halt the inflammatory response[51]. Human peroxisome proliferator-activated receptor contains three isoforms, as a result of alternative splicing and differential promoter usage within the same gene. PPARγ1, the predominant isoform, is expressed in a broad range of tissues, including small bowel and colon [52, 53]. PPARγ ligands have also been shown to markedly reduce colonic inflammation in both dextran sodium sulfate (DSS) and trinitrobenzene sulfonic acid colitis models in mice, probably through inhibition of NF-κB activity and decrease of proinflammatory cytokine production [54-57]. Conditional knockout of PPARγ enhanced tumorigenesis in ApcMin/1 mice [55].

Our preliminary studies show that administration of the selective sEH inhibitors, AUDA-nBE and *trans*-4-[4-(3-adamantan-1-ylureido) cyclohexyloxy]benzoic acid (t-AUCB) significantly inhibits colitis and ulcer formation in both DSS-induced ulcerative colitis (UC) mice and IL-10 knockout mice with spontaneous colitis. Immunohistochemical analysis revealed that AUDA-nBE treatment markedly suppressed myeloperoxidase-labeled inflammatory cell infiltration. Further mechanistic studies showed that AUDA-nBE induced peroxisome proliferator-activated receptor-gamma (PPAR-gamma) activity and its downstream gene expression such as Aquaporin-7, and inhibited NF-kB signaling. In addition, long-term administration of *t*-AUCB markedly inhibited the development of spontaneous colitis-induced dysplasia and carcinoma in IL-10 knockout mice.

The recent data indicate that EETs or sEH inhibitors not only regulate cytokines and chemokines but also regulate other key enzymes in the arachidonic acid cascade [1]. An increase in EETs results in a dramatic down regulation of induced, but not constitutive, COX-2 and 5-LOX and their associated metabolites leading to a reduction in symptoms associated with severe inflammation [1]. Simplistically the sEH inhibitors mediate an increase in EETs, resulting in a shift from eicosanoids initiating and propagating inflammation to a pattern of resolution of inflammation [1]. Thus, cross talk among the pathways in the arachidonate and other lipid cascades is likely to be a general phenomenon. *Patterns* of eicosanoid profiles are far more informative than *individua*l biomarkers or arachidonic acid metabolites, indicating that the eicosanoid profile could be a valuable biomarker for testing the effect of a sEH inhibitor [1, 39]. A simplistic plasma biomarker of sEH inhibitor effectiveness, which has held in multiple species, is the dramatic increase in the ratio of fatty acid epoxides to their corresponding diols. A more complex marker, which also yields mechanistic understanding, is a reduction in inflammatory COX and LOX metabolites in inflammatory models following the administration of sEH inhibitor.

In addition, the synergistic effect of sEH inhibitors and common NSAIDs is further studied in a lipopolysaccharide (LPS)-induced acute systemic inflammation model and shows that the sEH inhibitors not only are more potent than common NSAIDs in reducing predominantly inflammatory eicosanoids like prostaglandin PGE2 but they synergize with NSAIDs in reducing inflammation [1]. These effects can be anticipated from the mechanism of action where 1) the NSAIDs inhibit COX 1 and/or 2 and shift the arachidonic acid substrate toward other pathways including the LOX and P450 pathway. 2) The EETs stabilized by sEH inhibitors are directly anti-inflammatory, and transcriptionally reduce the COX 2 and 5-LOX induced by inflammation [58]. The sEH inhibitors also alter the eicosanoid profile associated with rapid blood clotting following administration of COX-2 inhibitors toward a more normal clotting response. Thus the sEH inhibitors should reduce the dose needed of some COX and LOX inhibitors for targeted therapy.

Development of highly potent and selective sEH inhibitors (sEHI)

The development of stable, highly potent, and highly selective inhibitors for sEH is a key and important step not only for the elucidation of the biology associated with EETs and sEH but also for developing a practical and efficient anti-inflammatory therapeutic agent. The sEHIs first identified were epoxides of either the substituted chalcone oxide or *trans*-3-phenylglycidol classes. Substituted chalcone oxides inhibit sEH through electronic stabilization of the covalent enzyme-inhibitor intermediate. *trans*-3-phenylglycidols bind sEH in an enantioselective manner and are slow sEHIs. Although these epoxide sEHIs are potent and selective, they are

unstable with transient sEH inhibitory effects [59, 60].

The newer class of sEHIs, including urea- and carbamate-based compounds, are much more potent and selective for sEH[60]. An example of this class of sEHIs is *N,N'*-dicyclohexylurea (DCU). DCU is weakly soluble in water, thus several urea-based sEHIs with increased water solubility and increased potency have been developed [61]. X-Ray crystal structure determinations show that the urea inhibitors establish hydrogen bonding and salt bridges between the urea portion of the inhibitor and residues on the sEH active site [7,62,63].

The most well studied sEH inhibitors are 12-(3-adamantyl-ureido)-dodecanoic acid (AUDA) based compounds. AUDA and its esters and salts have been widely used to evaluate the biological role of sEH. However, its rapid metabolism, moderate oral availability, and poor physical properties made this difficult. A typical modified agent in the AUDA family is 12-(3-adamantyl-ureido)-dodecanoic acid *n*-butyl ester (AUDA-*n*BE). AUDA was followed by the development of several sEHI compounds that bind within the catalytic site of sEH via hydrogen bonding. These compounds show dramatically improved water solubility, good oral bioavailability in multiple species, and have a lower melting point facilitating formulation [61, 62, 64-68]. Many structures with improved physical properties have resulted from placing hydroxyl, carbonyl, sulfonyl, ether, ester, carbamate, and other functionalities at the so-called secondary pharmacophore. Several N,N'-disubstituted urea compounds having a conformationally restricted *cis*- or *trans*-1,4-cyclohexane R group to the urea have been developed as sEH inhibitors with good oral bioavailability [61,62,64-68]. *Trans*-4-[4-(3-adamantan-1-ylureido)cyclohexyl oxy]benzoic acid (*t*-AUCB) is an example of this category of potent sEH inhibitors with a greater *in vivo* metabolic stability, low nanomolar activity (IC_{50} = 1.3 +/- 0.05nM) and increased oral bioavailability [64].

Potential role of sEH in inflammation-driven carcinogenesis

Non-steroidal anti-inflammatory drugs (NSAIDs) and selective COX-2 inhibitors have been shown to significantly reduce adenoma formation in patients with a high risk for the development of colorectal cancer. In 1988, Kune et al reported that aspirin use results in a decreased risk of developing colorectal carcinoma[69] and subsequent studies showed a decreased risk for the development of adenomatous polyps with aspirin use [70-73]. The development of COX-2 specific inhibitors seemed a promising option for the prevention of colorectal carcinoma without the toxicities seen with aspirin use, namely gastrointestinal bleeding. In the first randomized controlled clinical trial using a COX-2 inhibitor as chemoprevention for colorectal cancer, celecoxib was given to 83 patients with familial adenomatous polyposis (FAP) over a period of 6 months with a significant reduction in adenoma size and number and no recorded toxicities [74]. On the heels of this initial study, three major clinical trials were started investigating the effects of selective COX-2 inhibitors in patients at high-risk for colorectal carcinoma. The Adenoma Prevention with Celecoxib trial (APC) and the Prevention of Sporadic Adenomatous Polyps (PreSAP) trial both used celecoxib, while the Adenomatous Polyp Prevention on Vioxx (APPROVe) trial used rofecoxib [75-77]. All three trials found significant reductions in the development of adenomas, however all three trials were halted before completion due to an increased cardiovascular risk noted in patients participating in both the APPROVe and APC trials. The adverse endpoints in these two trials included myocardial infarction, stroke, congestive heart failure and death from cardiovascular disease [77-79]. In addition, the APPROVe trial showed that rofecoxib use was associated with an increased incidence of ulcers, bleeding, perforations and obstruction of the upper gastrointestinal tract [77].

Toxicity is a major concern in the development of anti-inflammatory agents, as is illustrated by the side effects of COX-2 inhibitors, such as rofecoxib. The thrombotic events associated with the use of rofecoxib and other NSAIDs have been attributed to a high dose but also to the destabilization of platelets due to the increased ratio of thromboxane A2 (TXA2) to prostacyclin (PGI2). However, sEH inhibitors have demonstrated cardiovascular protective effects in animal models of hypertension, and a phase I clinical trial is completed. For a phase I trial, one needs an investigational new drug approval (IND), which requires extensive toxicological studies that, in the case of SEHIs, to date are clean. In the academic laboratory, AUDA has

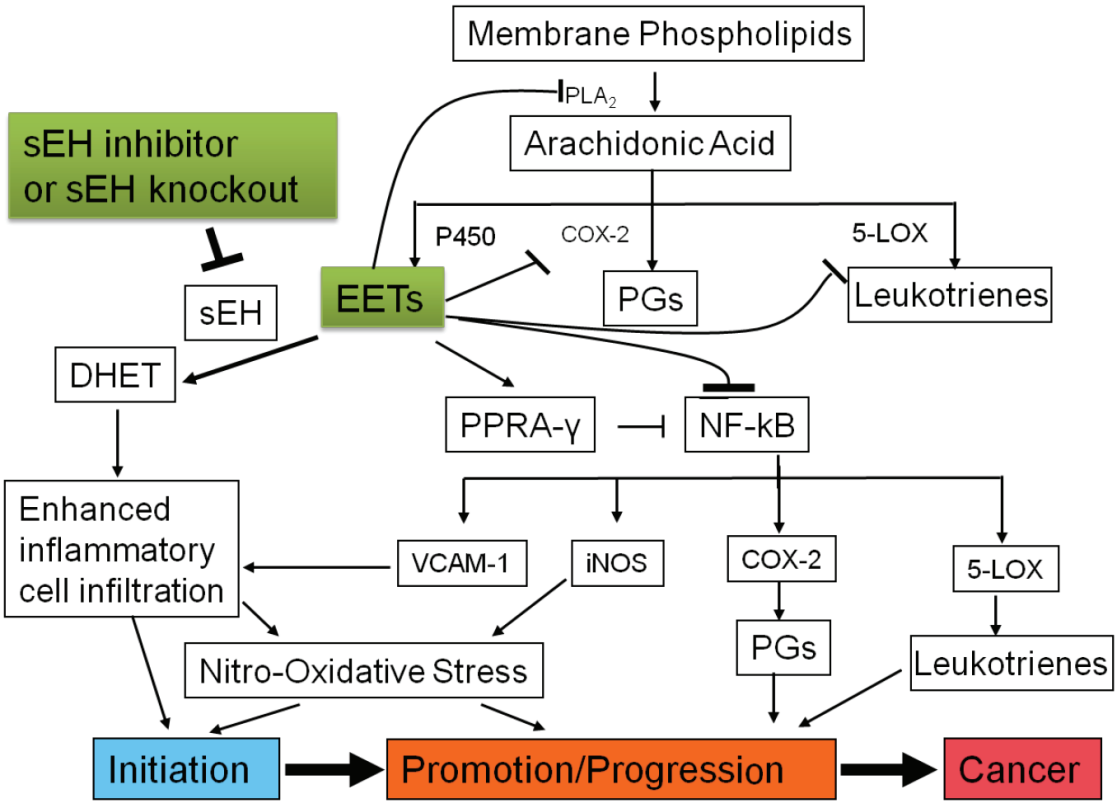

Figure 3. Multiple pathways of inflammation inhibition through targeting sEH. COX-2 = cyclooxygenase-2, DHETs = dihydroxyeicosatrienoic acids, EETs = epoxyeicosatrienoic acids, iNOS = inducible nitric oxide synthase, 5-LOX = 5-lipoxygenase, NF-κβ = nuclear factor- κβ, P450 = cytochrome P450, PGs = prostaglandins, PLA = phospholipase A, PPAR-γ = peroxisome proliferator-activated receptor-γ, VCAM-1 = vascular cell adhesion molecule-1.

shown high selectivity for sEH enzyme inhibition in a commercial screen of pharmacological and toxicological targets including COX and LOX enzymes. AUDA and *t*-AUCB have shown no obvious toxicity when tested at high oral doses in rodents.

There is limited information on the role of sEH in inflammation-induced carcinogenesis. Our preliminary study showed that up-regulation of sEH was identified in human ulcerative colitis (UC) and UC-induced dysplasia and carcinoma. Tissue microarrays of 180 patients with UC (n=72), colitis-induced dysplasia (n=54), and colitis-induced carcinoma (n=54) were established. Adjacent normal colonic tissue was also identified in these patients (n=79). Avidin-biotin-peroxidase approach was used with anti-sEH antibody and proper +/- controls. sEH staining positivity was evaluated for its intensity on a scale of 0-3+ and compared with positive controls(liver & kidney). sEH showed distinct expression in the microarray samples. Normal colon displayed positivity in 39.1% (n=79), mainly in the focally reactive epithelia. In UC, 74.6% (n=72) displayed positivity, extensively expressed in the hyperplastic epithelia. 88.2% (n=54) of colitis-induced dysplasia and 93.9% (n=54) of colitis-induced carcinoma displayed positivity. Markedly increased sEH staining intensity was observed in UC, UC-induced dysplasia and carcinoma and the average staining intensity of sEH was 0.43 in normal glands, 0.89 in UC, 1.29 in dysplasia, and 1.31 in carcinoma.

Conclusion

We believe that inhibition of sEH significantly reduces the conversion of EETs to their corresponding DHETs, and thus is a novel target for pharmacologic therapies of inflammation and

carcinogenesis. The mechanism of inflammation inhibition is by suppressing inflammatory cell recruitment, modulating the arachidonic acid metabolite profile and further targeting the PPAR-gamma and NF-kB pathways, which leads to an inhibition of COX-2, 5-LOX, iNOS, as well as VCAM-1 (**Figure 3**). Targeting of sEH with specific pharmacological inhibitors, as we have shown in animal models, provides direct experimental evidence for the role of sEH in inflammation and cancer development without the toxicities seen with the use of NSAIDs and selective COX-2 inhibitors. We believe sEH could be a novel biomarker for inflammation and inflammation-induced carcinogenesis and that sEH inhibitors could be used for cancer prevention with the same preventive effects of NSAIDs but without the adverse effects.

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References

[1] Inceoglu B, Schmelzer KR, Morisseau C, Jinks SL and Hammock BD. Soluble epoxide hydrolase inhibition reveals novel biological functions of epoxyeicosatrienoic acids (EETs). Prostaglandins Other Lipid Mediat 2007; 82: 42-49.

[2] Fretland AJ and Omiecinski CJ. Epoxide hydrolases: biochemistry and molecular biology. Chemico-Biological Interactions 2000; 129: 41-59.

[3] Sandberg M and Meijer J. Structural characterization of the human soluble epoxide hydrolase gene (EPHX2). Biochem Biophys Res Commun 1996; 221: 333-339.

[4] Larsson C, White I, Johansson C, Stark A and Meijer J. Localization of the human soluble epoxide hydrolase gene (EPHX2) to chromosomal region 8p21-p12. Hum Genet 1995; 95: 356-358.

[5] Cronin A, Mowbray S, Durk H, Homburg S, Fleming I, Fisslthaler B, Oesch F and Arand M. The N -terminal domain of mammalian soluble epoxide hydrolase is a phosphatase. Proc Natl Acad Sci U S A 2003; 100: 1552-1557.

[6] Newman JW, Morisseau C, Harris TR and Hammock BD. The soluble epoxide hydrolase encoded by EPXH2 is a bifunctional enzyme with novel lipid phosphate phosphatase activity. Proc Natl Acad Sci U S A 2003; 100: 1558-1563.

[7] Argiriadi MA, Morisseau C, Hammock BD and Christianson DW. Detoxification of environmental mutagens and carcinogens: structure, mechanism, and evolution of liver epoxide hydrolase. Proc Natl Acad Sci U S A 1999; 96: 10637-10642.

[8] Yu Z, Huse LM, Adler P, Graham L, Ma J, Zeldin DC and Kroetz DL. Increased CYP2J expression and epoxyeicosatrienoic acid formation in spontaneously hypertensive rat kidney. Molecular Pharmacology 2000; 57: 1011-1020.

[9] Fang X, VanRollins M, Kaduce TL and Spector AA. Epoxyeicosatrienoic acid metabolism in arterial smooth muscle cells. Journal of Lipid Research 1995; 36: 1236-1246.

[10] Johansson C, Stark A, Sandberg M, Ek B, Rask L and Meijer J. Tissue specific basal expression of soluble murine epoxide hydrolase and effects of clofibrate on the mRNA levels in extrahepatic tissues and liver. Archives of Toxicology 1995; 70: 61-63.

[11] VanRollins M, Kaduce TL, Knapp HR and Spector AA. 14,15-Epoxyeicosatrienoic acid metabolism in endothelial cells. Journal of Lipid Research 1993; 34: 1931-1942.

[12] Hollinshead M and Meijer J. Immunocytochemical analysis of soluble epoxide hydrolase and catalase in mouse and rat hepatocytes demonstrates a peroxisomal localization before and after clofibrate treatment. European Journal of Cell Biology 1988; 46: 394-402.

[13] Eriksson AM, Zetterqvist MA, Lundgren B, Andersson K, Beije B and DePierre JW. Studies on the intracellular distributions of soluble epoxide hydrolase and of catalase by digitonin-permeabilization of hepatocytes isolated from control and clofibrate-treated mice. European Journal of Biochemistry 1991; 198: 471-476.

[14] Mullen RT, Trelease RN, Duerk H, Arand M, Hammock BD, Oesch F and Grant DF. Differential subcellular localization of endogenous and transfected soluble epoxide hydrolase in mammalian cells: evidence for isozyme variants. FEBS Letters 1999; 445: 301-305.

[15] Enayetallah AE, French RA, Barber M and Grant DF. Cell-specific subcellular localization of soluble epoxide hydrolase in human tissues. J Histochem Cytochem 2006; 54: 329-335.

[16] Capdevila JH, Falck JR and Harris RC. Cytochrome P450 and arachidonic acid bioactivation. Molecular and functional properties of the arachidonate monooxygenase. Journal of Lipid Research 2000; 41: 163-181.

[17] Capdevila JH and Falck JR. The CYP P450 arachidonic acid monooxygenases: from cell signaling to blood pressure regulation. Biochemical & Biophysical Research Communications 2001; 285: 571-576.

[18] Karara A, Dishman E, Falck JR and Capdevila JH. Endogenous epoxyeicosatrienoyl-phospholipids. A novel class of cellular glycerolipids containing epoxidized arachidonate moie-

ties. Journal of Biological Chemistry 1991; 266: 7561-7569.
[19] Karara A, Dishman E, Jacobson H, Falck JR and Capdevila JH. Arachidonic acid epoxygenase. Stereochemical analysis of the endogenous epoxyeicosatrienoic acids of human kidney cortex. FEBS Letters 1990; 268: 227-230.
[20] Karara A, Wei S, Spady D, Swift L, Capdevila JH and Falck JR. Arachidonic acid epoxygenase: structural characterization and quantification of epoxyeicosatrienoates in plasma. Biochemical & Biophysical Research Communications 1992; 182: 1320-1325.
[21] Weintraub NL, Fang X, Kaduce TL, VanRollins M, Chatterjee P and Spector AA. Epoxide hydrolases regulate epoxyeicosatrienoic acid incorporation into coronary endothelial phospholipids. American Journal of Physiology 1999; 277: H2098-2108.
[22] Roman RJ and Roman RJ. P-450 metabolites of arachidonic acid in the control of cardiovascular function. Physiological Reviews 2002; 82: 131-185.
[23] Spector AA, Fang X, Snyder GD, Weintraub NL, Spector AA, Fang X, Snyder GD and Weintraub NL. Epoxyeicosatrienoic acids (EETs): metabolism and biochemical function. Progress in Lipid Research 2004; 43: 55-90.
[24] Li PL and Campbell WB. Epoxyeicosatrienoic acids activate K+ channels in coronary smooth muscle through a guanine nucleotide binding protein. Circulation Research 1997; 80: 877-884.
[25] Fukao M, Mason HS, Kenyon JL, Horowitz B and Keef KD. Regulation of BK(Ca) channels expressed in human embryonic kidney 293 cells by epoxyeicosatrienoic acid. Molecular Pharmacology 2001; 59: 16-23.
[26] Node K, Ruan XL, Dai J, Yang SX, Graham L, Zeldin DC and Liao JK. Activation of Galpha s mediates induction of tissue-type plasminogen activator gene transcription by epoxyeicosatrienoic acids. Journal of Biological Chemistry 2001; 276: 15983-15989.
[27] Chen JK, Capdevila J and Harris RC. Overexpression of C-terminal Src kinase blocks 14, 15 -epoxyeicosatrienoic acid-induced tyrosine phosphorylation and mitogenesis. Journal of Biological Chemistry 2000; 275: 13789-13792.
[28] Hoebel BG and Graier WF. 11,12-Epoxyeicosatrienoic acid stimulates tyrosine kinase activity in porcine aortic endothelial cells. European Journal of Pharmacology 1998; 346: 115-117.
[29] Seubert J, Yang B, Bradbury JA, Graves J, Degraff LM, Gabel S, Gooch R, Foley J, Newman J, Mao L, Rockman HA, Hammock BD, Murphy E, Zeldin DC, Seubert J, Yang B, Bradbury JA, Graves J, Degraff LM, Gabel S, Gooch R, Foley J, Newman J, Mao L, Rockman HA, Hammock BD, Murphy E and Zeldin DC. Enhanced postischemic functional recovery in CYP2J2 transgenic hearts involves mitochondrial ATP-sensitive K+ channels and p42/p44 MAPK pathway.[see comment]. Circulation Research 2004; 95: 506-514.
[30] Wang Y, Wei X, Xiao X, Hui R, Card JW, Carey MA, Wang DW, Zeldin DC, Wang Y, Wei X, Xiao X, Hui R, Card JW, Carey MA, Wang DW and Zeldin DC. Arachidonic acid epoxygenase metabolites stimulate endothelial cell growth and angiogenesis via mitogen-activated protein kinase and phosphatidylinositol 3-kinase/Akt signaling pathways. Journal of Pharmacology & Experimental Therapeutics 2005; 314: 522-532.
[31] Fang X, Kaduce TL, VanRollins M, Weintraub NL and Spector AA. Conversion of epoxyeicosatrienoic acids (EETs) to chain-shortened epoxy fatty acids by human skin fibroblasts. Journal of Lipid Research 2000; 41: 66-74.
[32] Fang X, Kaduce TL, Weintraub NL, Harmon S, Teesch LM, Morisseau C, Thompson DA, Hammock BD and Spector AA. Pathways of epoxyeicosatrienoic acid metabolism in endothelial cells. Implications for the vascular effects of soluble epoxide hydrolase inhibition. Journal of Biological Chemistry 2001; 276: 14867-14874.
[33] Fang X, Kaduce TL, Weintraub NL, VanRollins M and Spector AA. Functional implications of a newly characterized pathway of 11,12-epoxyeicosatrienoic acid metabolism in arterial smooth muscle. Circulation Research 1996; 79: 784-793.
[34] Cowart LA, Wei S, Hsu MH, Johnson EF, Krishna MU, Falck JR, Capdevila JH, Cowart LA, Wei S, Hsu M-H, Johnson EF, Krishna MU, Falck JR and Capdevila JH. The CYP4A isoforms hydroxylate epoxyeicosatrienoic acids to form high affinity peroxisome proliferator-activated receptor ligands. Journal of Biological Chemistry 2002; 277: 35105-35112.
[35] Carroll MA, Balazy M, Margiotta P, Falck JR and McGiff JC. Renal vasodilator activity of 5,6-epoxyeicosatrienoic acid depends upon conversion by cyclooxygenase and release of prostaglandins. Journal of Biological Chemistry 1993; 268: 12260-12266.
[36] Zhang JY, Prakash C, Yamashita K and Blair IA. Regiospecific and enantioselective metabolism of 8,9-epoxyeicosatrienoic acid by cyclooxygenase. Biochemical & Biophysical Research Communications 1992; 183: 138-143.
[37] Pace-Asciak CR, Granstrom E and Samuelsson B. Arachidonic acid epoxides. Isolation and structure of two hydroxy epoxide intermediates in the formation of 8,11,12- and 10,11,12-trihydroxyeicosatrienoic acids. Journal of Biological Chemistry 1983; 258: 6835-6840.
[38] Spearman ME, Prough RA, Estabrook RW, Falck JR, Manna S, Leibman KC, Murphy RC and Capdevila J. Novel glutathione conjugates formed

from epoxyeicosatrienoic acids (EETs). Archives of Biochemistry & Biophysics 1985; 242: 225-230.
[39] Chiamvimonvat N, Ho CM, Tsai HJ and Hammock BD. The soluble epoxide hydrolase as a pharmaceutical target for hypertension. J Cardiovasc Pharmacol 2007; 50: 225-237.
[40] Huang H, Morisseau C, Wang J, Yang T, Falck JR, Hammock BD and Wang MH. Increasing or stabilizing renal epoxyeicosatrienoic acid production attenuates abnormal renal function and hypertension in obese rats. Am J Physiol Renal Physiol 2007; 293: F342-349.
[41] Zhang W, Koerner IP, Noppens R, Grafe M, Tsai HJ, Morisseau C, Luria A, Hammock BD, Falck JR and Alkayed NJ. Soluble epoxide hydrolase: a novel therapeutic target in stroke. J Cereb Blood Flow Metab 2007; 27: 1931-1940.
[42] Schmelzer KR, Kubala L, Newman JW, Kim IH, Eiserich JP and Hammock BD. Soluble epoxide hydrolase is a therapeutic target for acute inflammation. Proc Natl Acad Sci U S A 2005; 102: 9772-9777.
[43] Smith KR, Pinkerton KE, Watanabe T, Pedersen TL, Ma SJ and Hammock BD. Attenuation of tobacco smoke-induced lung inflammation by treatment with a soluble epoxide hydrolase inhibitor. Proc Natl Acad Sci U S A 2005; 102: 2186-2191.
[44] Zhao X, Yamamoto T, Newman JW, Kim IH, Watanabe T, Hammock BD, Stewart J, Pollock JS, Pollock DM and Imig JD. Soluble epoxide hydrolase inhibition protects the kidney from hypertension-induced damage. J Am Soc Nephrol 2004; 15: 1244-1253.
[45] Node K, Huo Y, Ruan X, Yang B, Spiecker M, Ley K, Zeldin DC and Liao JK. Anti-inflammatory properties of cytochrome P450 epoxygenase-derived eicosanoids. Science 1999; 285: 1276-1279.
[46] Fleming I. DiscrEET regulators of homeostasis: epoxyeicosatrienoic acids, cytochrome P450 epoxygenases and vascular inflammation. Trends Pharmacol Sci 2007; 28: 448-452.
[47] Inceoglu B, Jinks SL, Schmelzer KR, Waite T, Kim IH and Hammock BD. Inhibition of soluble epoxide hydrolase reduces LPS-induced thermal hyperalgesia and mechanical allodynia in a rat model of inflammatory pain. Life Sci 2006; 79: 2311-2319.
[48] Liu Y, Zhang Y, Schmelzer K, Lee TS, Fang X, Zhu Y, Spector AA, Gill S, Morisseau C, Hammock BD and Shyy JY. The antiinflammatory effect of laminar flow: the role of PPARgamma, epoxyeicosatrienoic acids, and soluble epoxide hydrolase. Proc Natl Acad Sci U S A 2005; 102: 16747-16752.
[49] Ward JE and Tan X. Peroxisome proliferator activated receptor ligands as regulators of airway inflammation and remodelling in chronic lung disease. PPAR Res 2007; 2007: 14983.
[50] Stienstra R, Duval C, Muller M and Kersten S. PPARs, Obesity, and Inflammation. PPAR Res 2007; 2007: 95974.
[51] Dubuquoy L, Rousseaux C, Thuru X, Peyrin-Biroulet L, Romano O, Chavatte P, Chamaillard M and Desreumaux P. PPARgamma as a new therapeutic target in inflammatory bowel diseases. Gut 2006; 55: 1341-1349.
[52] Auboeuf D, Rieusset J, Fajas L, Vallier P, Frering V, Riou JP, Staels B, Auwerx J, Laville M and Vidal H. Tissue distribution and quantification of the expression of mRNAs of peroxisome proliferator-activated receptors and liver X receptor -alpha in humans: no alteration in adipose tissue of obese and NIDDM patients. Diabetes 1997; 46: 1319-1327.
[53] Fajas L, Auboeuf D, Raspe E, Schoonjans K, Lefebvre AM, Saladin R, Najib J, Laville M, Fruchart JC, Deeb S, Vidal-Puig A, Flier J, Briggs MR, Staels B, Vidal H and Auwerx J. The organization, promoter analysis, and expression of the human PPARgamma gene. J Biol Chem 1997; 272: 18779-18789.
[54] Ramakers JD, Verstege MI, Thuijls G, Te Velde AA, Mensink RP and Plat J. The PPARgamma agonist rosiglitazone impairs colonic inflammation in mice with experimental colitis. J Clin Immunol 2007; 27: 275-283.
[55] McAlpine CA, Barak Y, Matise I and Cormier RT. Intestinal-specific PPARgamma deficiency enhances tumorigenesis in ApcMin/+ mice. Int J Cancer 2006; 119: 2339-2346.
[56] Ponferrada A, Caso JR, Alou L, Colon A, Sevillano D, Moro MA, Lizasoain I, Menchen P, Gomez-Lus ML, Lorenzo P, Cos E, Leza JC and Menchen L. The role of PPARgamma on restoration of colonic homeostasis after experimental stress-induced inflammation and dysfunction. Gastroenterology 2007; 132: 1791-1803.
[57] Kohno H, Suzuki R, Sugie S and Tanaka T. Suppression of colitis-related mouse colon carcinogenesis by a COX-2 inhibitor and PPAR ligands. BMC Cancer 2005; 5: 46.
[58] Schmelzer KR, Inceoglu B, Kubala L, Kim IH, Jinks SL, Eiserich JP and Hammock BD. Enhancement of antinociception by coadministration of nonsteroidal anti-inflammatory drugs and soluble epoxide hydrolase inhibitors. Proc Natl Acad Sci U S A 2006; 103: 13646-13651.
[59] Morisseau C, Du G, Newman JW and Hammock BD. Mechanism of mammalian soluble epoxide hydrolase inhibition by chalcone oxide derivatives. Archives of Biochemistry & Biophysics 1998; 356: 214-228.
[60] Morisseau C, Goodrow MH, Dowdy D, Zheng J, Greene JF, Sanborn JR and Hammock BD. Potent urea and carbamate inhibitors of soluble epoxide hydrolases. Proc Natl Acad Sci U S A 1999; 96: 8849-8854.
[61] Morisseau C, Goodrow MH, Newman JW, Wheelock CE, Dowdy DL and Hammock BD. Structural refinement of inhibitors of urea-based soluble epoxide hydrolases. Biochem Pharma-

col 2002; 63: 1599-1608.
[62] Kim IH, Morisseau C, Watanabe T and Hammock BD. Design, synthesis, and biological activity of 1,3-disubstituted ureas as potent inhibitors of the soluble epoxide hydrolase of increased water solubility. J Med Chem 2004; 47: 2110-2122.
[63] Argiriadi MA, Morisseau C, Goodrow MH, Dowdy DL, Hammock BD and Christianson DW. Binding of alkylurea inhibitors to epoxide hydrolase implicates active site tyrosines in substrate activation. J Biol Chem 2000; 275: 15265-15270.
[64] Hwang SH, Tsai HJ, Liu JY, Morisseau C and Hammock BD. Orally bioavailable potent soluble epoxide hydrolase inhibitors. J Med Chem 2007; 50: 3825-3840.
[65] Kim IH, Nishi K, Tsai HJ, Bradford T, Koda Y, Watanabe T, Morisseau C, Blanchfield J, Toth I and Hammock BD. Design of bioavailable derivatives of 12-(3-adamantan-1-yl-ureido) dodecanoic acid, a potent inhibitor of the soluble epoxide hydrolase. Bioorg Med Chem 2007; 15: 312-323.
[66] Wolf NM, Morisseau C, Jones PD, Hock B and Hammock BD. Development of a high-throughput screen for soluble epoxide hydrolase inhibition. Anal Biochem 2006; 355: 71-80.
[67] Gomez GA, Morisseau C, Hammock BD and Christianson DW. Human soluble epoxide hydrolase: structural basis of inhibition by 4-(3-cyclohexylureido)-carboxylic acids. Protein Sci 2006; 15: 58-64.
[68] Kim IH, Heirtzler FR, Morisseau C, Nishi K, Tsai HJ and Hammock BD. Optimization of amide-based inhibitors of soluble epoxide hydrolase with improved water solubility. J Med Chem 2005; 48: 3621-3629.
[69] Kune GA, Kune S and Watson LF. Colorectal cancer risk, chronic illnesses, operations, and medications: case control results from the Melbourne Colorectal Cancer Study. Cancer Research 1988; 48: 4399-4404.
[70] Suh O, Mettlin C and Petrelli NJ. Aspirin use, cancer, and polyps of the large bowel. Cancer 1993; 72: 1171-1177.
[71] Greenberg ER, Baron JA, Freeman DH, Jr., Mandel JS and Haile R. Reduced risk of large-bowel adenomas among aspirin users. The Polyp Prevention Study Group. Journal of the National Cancer Institute 1993; 85: 912-916.
[72] Logan RF, Little J, Hawtin PG and Hardcastle JD. Effect of aspirin and non-steroidal anti-inflammatory drugs on colorectal adenomas: case-control study of subjects participating in the Nottingham faecal occult blood screening programme.[see comment]. BMJ 1993; 307: 285-289.
[73] Martinez ME, McPherson RS, Levin B and Annegers JF. Aspirin and other nonsteroidal anti-inflammatory drugs and risk of colorectal adenomatous polyps among endoscoped individuals. Cancer Epidemiology, Biomarkers & Prevention 1995; 4: 703-707.
[74] Steinbach G, Lynch PM, Phillips RK, Wallace MH, Hawk E, Gordon GB, Wakabayashi N, Saunders B, Shen Y, Fujimura T, Su LK and Levin B. The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. New England Journal of Medicine 2000; 342: 1946-1952.
[75] Bertagnolli MM, Eagle CJ, Zauber AG, Redston M, Solomon SD, Kim K, Tang J, Rosenstein RB, Wittes J, Corle D, Hess TM, Woloj GM, Boisserie F, Anderson WF, Viner JL, Bagheri D, Burn J, Chung DC, Dewar T, Foley TR, Hoffman N, Macrae F, Pruitt RE, Saltzman JR, Salzberg B, Sylwestrowicz T, Gordon GB, Hawk ET, Investigators APCS, Bertagnolli MM, Eagle CJ, Zauber AG, Redston M, Solomon SD, Kim K, Tang J, Rosenstein RB, Wittes J, Corle D, Hess TM, Woloj GM, Boisserie F, Anderson WF, Viner JL, Bagheri D, Burn J, Chung DC, Dewar T, Foley TR, Hoffman N, Macrae F, Pruitt RE, Saltzman JR, Salzberg B, Sylwestrowicz T, Gordon GB and Hawk ET. Celecoxib for the prevention of sporadic colorectal adenomas.[see comment]. New England Journal of Medicine 2006; 355: 873-884.
[76] Arber N, Eagle CJ, Spicak J, Racz I, Dite P, Hajer J, Zavoral M, Lechuga MJ, Gerletti P, Tang J, Rosenstein RB, Macdonald K, Bhadra P, Fowler R, Wittes J, Zauber AG, Solomon SD, Levin B, Pre SAPTI, Arber N, Eagle CJ, Spicak J, Racz I, Dite P, Hajer J, Zavoral M, Lechuga MJ, Gerletti P, Tang J, Rosenstein RB, Macdonald K, Bhadra P, Fowler R, Wittes J, Zauber AG, Solomon SD and Levin B. Celecoxib for the prevention of colorectal adenomatous polyps.[see comment]. New England Journal of Medicine 2006; 355: 885-895.
[77] Baron JA, Sandler RS, Bresalier RS, Quan H, Riddell R, Lanas A, Bolognese JA, Oxenius B, Horgan K, Loftus S, Morton DG, Investigators APT, Baron JA, Sandler RS, Bresalier RS, Quan H, Riddell R, Lanas A, Bolognese JA, Oxenius B, Horgan K, Loftus S and Morton DG. A randomized trial of rofecoxib for the chemoprevention of colorectal adenomas.[see comment]. Gastroenterology 2006; 131: 1674-1682.
[78] Bresalier RS, Sandler RS, Quan H, Bolognese JA, Oxenius B, Horgan K, Lines C, Riddell R, Morton D, Lanas A, Konstam MA, Baron JA, Adenomatous Polyp Prevention on Vioxx Trial I, Bresalier RS, Sandler RS, Quan H, Bolognese JA, Oxenius B, Horgan K, Lines C, Riddell R, Morton D, Lanas A, Konstam MA and Baron JA. Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial.[see comment][erratum appears in N Engl J Med. 2006 Jul 13;355(2):221]. New England Journal of Medicine 2005; 352: 1092-1102.

[79] Solomon SD, McMurray JJ, Pfeffer MA, Wittes J, Fowler R, Finn P, Anderson WF, Zauber A, Hawk E, Bertagnolli M, Adenoma Prevention with Celecoxib Study I, Solomon SD, McMurray JJV, Pfeffer MA, Wittes J, Fowler R, Finn P, Anderson WF, Zauber A, Hawk E and Bertagnolli M. Cardiovascular risk associated with celecoxib in a clinical trial for colorectal adenoma prevention. [see comment]. New England Journal of Medicine 2005; 352: 1071-1080.