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Review Article

Soluble Epoxide Hydrolase

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A B S T R A C T

Epoxyeicosatrienoic acids (EETs) have numerous cardiovascular benefits, including vasodilation, anti-inflammatory actions, and anti-migratory effects on vascular smooth muscle cells. However, sEH, an enzyme that breaks down EETs into diols, limits these benefits. The development of sEH inhibitors (sEHIs), particularly those based on 1,3-disubstituted urea, has shown promise in enhancing the therapeutic properties of EETs. These inhibitors are antihypertensive and anti-inflammatory and can protect the heart, brain, and kidneys from damage. While there are still challenges to overcome, such as improving the drug-like properties of sEHIs and finding better ways to target specific tissues, the initiation of clinical trials for sEHIs highlights their potential as therapeutic agents.

Keywords: - Soluble Epoxide Hydrolase, Epoxyeicosatrienoic acids, Arachidonic Acid, Leukotriene.

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INTRODUCTION

The arachidonate cascade has many enzymes, receptors, and eicosanoid metabolites that can be used to treat inflammatory diseases. The first targeted pathway was the cyclooxygenase (COX) pathway, responsible for producing prostaglandins. Aspirin and non-steroidal anti-inflammatory drugs (NSAIDs) are effective pain and inflammation relievers, including inhibitors of COX2 (also known as PTGS2). These drugs are also useful for treating or preventing cardiovascular disease [1]. Aspirin can reduce the risk of ischemic events such as heart attacks and strokes by inhibiting blood clotting. Prostacyclin analogues are used for the treatment of pulmonary hypertension [2]. However, COX2 inhibitors increased the incidence of acute renal failure, myocardial infarction, and thrombotic stroke in patients, which diminished enthusiasm for targeting the COX pathway. The second eicosanoid and inflammatory pathway was the generation of leukotrienes by lipoxygenase (LOX). Arachidonate 5 lipoxygenase (ALOX5) and leukotriene receptor antagonists were developed to treat asthma and seasonal allergies. These two eicosanoid pathways have become increasingly important therapeutic targets as novel receptors and metabolites are identified, and their roles in many diseases are better defined.1980 a third eicosanoid pathway, known as cytochrome (CYP), was discovered. It is composed of two enzymatic pathways, namely hydroxylases, and epoxygenases, which convert arachidonic acid into hydroxyeicosatetraenoic acids (HETEs) and Epoxyeicosatrienoic acids (EETs) respectively [3,4]. The hydroxylase CYP enzymes convert arachidonic acid into HETEs, with 20-HETE being the primary metabolite. This pathway is pro-inflammatory and crucial to vascular function. It is currently being targeted for the treatment of cardiovascular diseases like hypertension and stroke [5]. On the other hand, the epoxygenase CYP enzymes generate EETs by catalysing the epoxidation of arachidonic acid olefin bonds, resulting in the production of four regioisomeric EETs, namely 5,6-EET, 8,9-EET, 11,12-EET,

and 14,15-EET. EETs are endothelium-derived hyperpolarizing factors (EDHFs) that protect against ischaemic injury and have anti-inflammatory properties in canine and rodent disease models [6]. However, sEH enzyme converts EETs to their corresponding diols (dihydroxyeicosatrienoic acids; DHETs), which decreases EET levels and diminishes their beneficial cardiovascular properties [7]. Thus, inhibiting this enzyme can be an effective therapeutic strategy for cardiovascular disease (As shown in fig no.1). Recently, sEH inhibitors (sEHIs) have been developed to enhance the cardiovascular actions of EETs [8]

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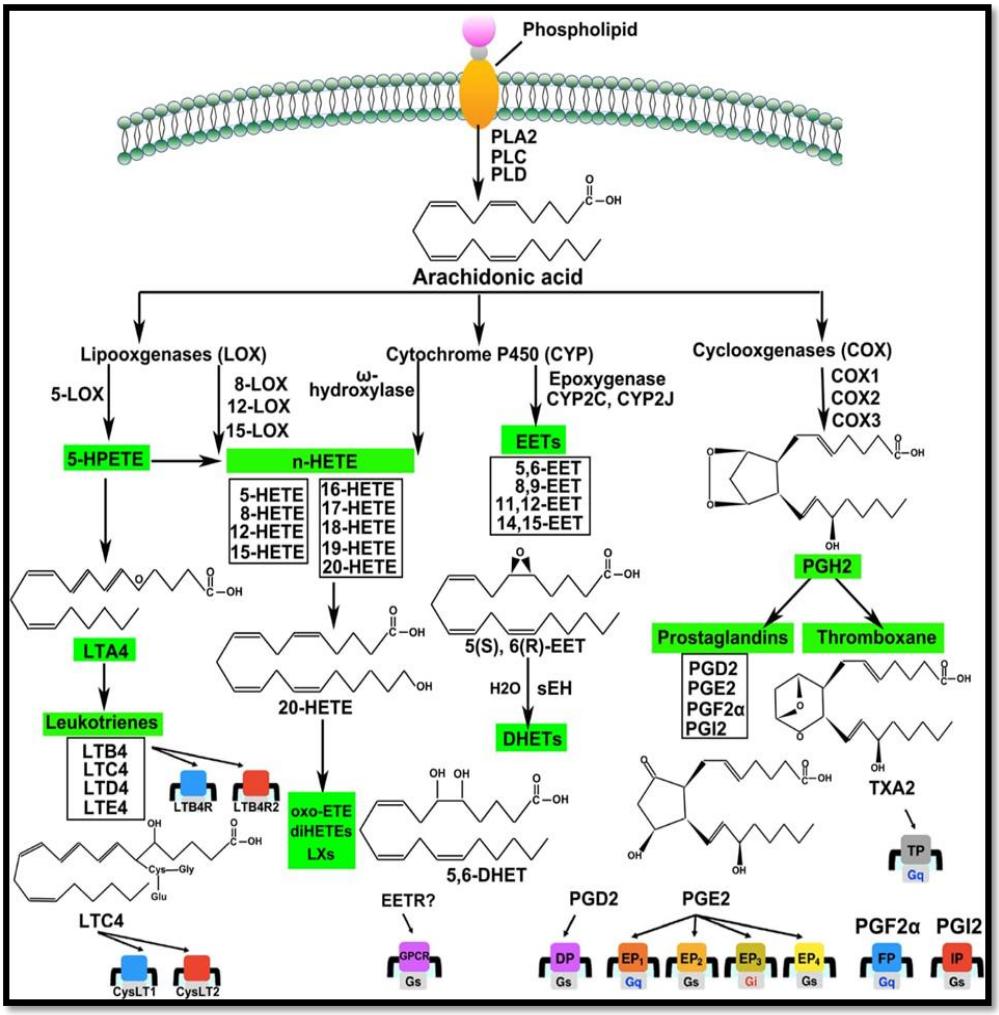

Figure No. 1: Arachidonic acid (AA) is metabolized through three pathways. Three main enzymes (PLA2, PLC, and PLD) release AA from membrane-bound phospholipids. PGHSs (COXs) produce protanoids, prostacyclin, and thromboxane, while LOXs produce leukotrienes and HETEs. P450 epoxygenases metabolize AA to midchain HETEs and four EET regioisomers, which are then further metabolized to less active DHETs by sEH.

Biological action: -

Since the first descriptions of the biological actions of EETs, which included increases in epithelial transport in the kidney and dilation of small mesenteric resistance arteries, there has been growing interest in these eicosanoid metabolites. Interest in EETs was greatly increased in 1996 after their identification as EDHFs. Over the past decade, it has become increasingly apparent that EETs have many cardiovascular actions, most of which seem to be cardiovascular protective. However, the cellular signaling mechanisms responsible for the biological actions of EETs continue to be intensely investigated [9].

There is ample evidence that EETs bind to receptors that are coupled by a G protein to intracellular signaling cascades. However, an EET receptor has yet to be identified. EETs could also function inside the cell by coupling to and activating ion channels, signaling proteins or transcription factors. Experimental evidence supports an intracellular mechanism of action, in that EETs are incorporated into cell membrane phospholipids and bind to fatty-acid-binding proteins and peroxisome proliferator-activated receptor-γ (PPARγ)[10].

The biological activities and cellular signaling mechanisms of EETs have been comprehensively reviewed elsewhere. As with other eicosanoid pathways, the cellular signaling mechanisms and biological activities of EETs vary depending on the cell type and tissue. Other experimental issues have made it difficult to investigate EETs and the CYP enzymatic pathways. The common concerns relate to the quality and purity of regioisomeric EETs and the correct method of using them.

Similarly, investigations in cell culture systems are limited by the fact that the levels of epoxygenase and epoxide hydrolase enzymes decrease rapidly following cell isolation. Experimental approaches to circumvent these issues include the generation of genetically manipulated mice, transfection of cell culture lines with CYP enzymes, and the development of EET analogues and antagonists that have improved chemical properties and greater stability[11].

Moreover, EET receptor identification could help to clarify the apparent biological heterogeneity of EET signaling, much like the discovery of multiple prostaglandin E2 (PGE2) receptors helped explain the apparent contradictions in biological and cell signaling mechanisms of PGE2. Despite these experimental concerns, there has been tremendous progress in determining EET biological actions, and EETs remain an attractive therapeutic target for cardiovascular diseases [12].

Vascular action: -

EETs have been extensively studied for their cardiovascular actions, particularly as vasodilators and endothelium-derived hyperpolarizing factors. EETs have been found to cause vasodilation in organs such as the heart, brain, kidney, skeletal muscle and intestine. However, in the lung, EETs cause vasoconstriction, which was expected due to the opposite effects of prostaglandins in this vasculature [13]. All regioisomeric EETs are vasodilators, with 11,12-EET and 14,15-EET exhibiting the greatest vasodilator activity. These two regioisomeric EETs are generated by endothelial cells and dilate blood vessels by activating large-conductance Ca2+-activated K+ (KCa) channels on vascular smooth muscle cells. This results in K+ efflux from the smooth muscle cell and subsequent membrane hyperpolarization [14]. EETs activate KCa channels and dilate blood vessels by cyclic AMP activation of protein kinase A (PKA) and ADP ribosylation of the α-subunit of the stimulatory G protein (Gs α). The conversion of EETs to DHETs by sEH can regulate the ability of EETs to activate KCa channels and dilate blood vessels. DHETs have little or no ability to cause vasorelaxation, which is why sEH inhibition improves dilator activity in human blood vessels [15]. EETs or sEHIs oppose the vasoconstrictor activities of the pro-hypertensive hormones endothelin 1 and angiotensin II. Therefore, decreased endothelial EET conversion to DHETs could be one mechanism responsible for the antihypertensive actions of sEHIs, as well as for their other cardio protective properties [16].

Endothelial cell and vascular smooth muscle cell proliferation and migration are regulated by EETs and sEH. EETs promote angiogenesis and endothelial cell proliferation and migration. Epoxides cause the proliferative effects, not the corresponding diols. EETs or overexpression of CYP2C epoxygenases lead to proliferative responses, which are attributed to the activation of two cell signaling pathways. The p38 mitogen-activated protein kinase (MAPK) pathway and the phosphatidyl-inositol 3-kinase–AKT (PI3K–AKT) pathway are both activated by EETs. 11,12-EET activates MAPK, which upregulates cyclin D and AKT. AKT then phosphorylates forkhead factors and decreases the expression of the cyclin-dependent kinase inhibitor p27kip1 in endothelial cells [17]. 11,12-EET-mediated proliferation, migration, and tube formation in human umbilical vein cells are dependent on activation of sphingosine kinase 1. Sphingosine kinase 1 phosphorylates sphingosine to generate sphingosine-1-phosphate (S1P). EETs have antimigratory actions in vascular smooth muscle cells. 11,12-EET and 14,15-EET moderately attenuated the migration of aortic smooth muscle cells in response to platelet-derived growth factor. Overexpression of CYP2J epoxygenase or inhibition of sEH also reduced smooth muscle cell proliferation and migration. Activation of the cAMP-dependent PKA pathway and decreased cyclin D levels have been implicated in the antimigratory actions of EETs and sEHIs in vascular smooth muscle cells [18].

While some reports suggest an effect on vascular smooth muscle cells by EETs and sEHIs, other studies have failed to show that EETs or sEHIs cause vascular smooth muscle proliferation. Nonetheless, the effects of EETs and sEHIs on the proliferation and migration of endothelial and vascular smooth muscle cells indicate the importance of targeting this pathway in angiogenesis, atherosclerosis, and other cardiovascular diseases. EETs are important regulators of vascular homeostasis, and sEH seems to play a crucial role in the regulation of EET actions.

Anti-inflammatory actions of EETs: -

Inflammatory diseases can contribute significantly to damage to blood vessels, organs, and the progression of cardiovascular disease. The epoxygenase pathway can also affect cardiovascular function in disease states and interact with inflammation. Cytokines can decrease CYP2C expression and oppose epoxygenase-mediated vasodilation. Conversely, inhibition of TNF or CC-chemokine receptor 2 can increase kidney CYP2C expression and decrease renal injury in hypertension [19].

Experimental evidence suggests that EETs interfere with the activation of the transcription factor nuclear factor κB (NF-κB) and exert their vascular anti-inflammatory effects (As shown in fig no.3). 11,12-EET is particularly effective at preventing TNF-induced activation of NF-κB and at increasing the expression of vascular cell adhesion molecule 1 (VCAM1) in endothelial cells. Similarly, CYP2J epoxygenase overexpression in endothelial cells decreased NF-κB activation. Although further research is required to determine the exact cellular signalling mechanisms responsible for the anti-inflammatory actions of EETs, it is clear that they decrease inflammation [20].

EETs also have other anti-inflammatory actions, including decreasing the aggregation of human polymorphonuclear leukocytes and decreasing leukocyte adhesion to endothelial cells. They also decreased the fever induced by interleukin-1β, with 11,12-EET being particularly effective. Studies using sEHIs also support the notion that EETs have anti-inflammatory actions, which could be protective against the deleterious effects of inflammation associated with cardiovascular diseases. They could also provide treatment for other inflammatory diseases [21].

Design of sEHIs: -

In the past decade, there has been a remarkable development of soluble epoxide hydrolase inhibitors (sEHIs) for in vivo use and clinical testing. A significant study conducted in

2000 (Ref. 22) demonstrated that the injection of an sEHI to the spontaneously hypertensive rat (SHR) reduced blood pressure. This study was followed by the first evidence that chronic inhibition of sEH also lowered blood pressure in angiotensin-induced hypertension. Another breakthrough came in 2005, when an orally administered sEHI was shown to be antihypertensive and slowed the progression of renal damage. Since then, a number of studies have revealed exciting findings about the broad potential for sEHIs as cardiovascular therapeutic agents, and a first-in-class sEHI began clinical Phase IIa testing this year (Arete Therapeutics Initiates Phase IIa clinical trial for AR9281, a novel sEH inhibitor to treat type 2 diabetes [22,23].

In this section, we describe the evolution of selective sEHIs, from enzyme inhibition in vitro to oral administration in rodents and subsequently humans. There are two well-studied α–β-hydrolase fold epoxide hydrolase enzymes that differ by subcellular localization and substrate selectivity. The microsomal epoxide hydrolase (mEH) is involved in the metabolism of environmental contaminants and has been extensively studied in this role. The sEH was initially discovered in studies on the metabolism of a terpenoid epoxide that mimicked insect juvenile hormone. At the same time, EETs were being established as endogenous lipid mediators with biological activity. It was subsequently discovered that arachidonic acid and linoleic acid epoxides are metabolized by sEH, and sEH converts these epoxides to diols with high Vmax and low Km. The sEH gene and transcript have been cloned recently and the structure and catalytic mechanism of sEH have been determined. Mammalian sEH is composed of two domains, the carboxy-terminal domain and the amino-terminal domain, which have epoxide hydrolase and phosphatase activities respectively. The enzyme is widely distributed in tissues, with highest activity in the liver and kidney. The function of the N-terminal domain of sEH is still unclear, but it has been suggested that it could stabilize the epoxide hydrolase activity and promote dimerization of the sEH enzyme. Current sEHIs inhibit the epoxide hydrolase activity of the C-terminal domain without affecting the phosphatase activity of the N-terminal domain. First-generation sEHIs were potent competitive inhibitors, but they were rapidly inactivated by glutathione and glutathione transferases, making them difficult to use in tissue samples and in vivo. Later, amides, ureas and carbamates were found to be potent and stable transition state inhibitors of sEH, facilitating experiments to investigate the endogenous roles of this enzyme. These inhibitors inhibit the C-terminal epoxide hydrolase activity of the sEH enzyme with nanomolar Ki values but do not substantially alter the phosphatase activity of the N-terminal domain. The design of these inhibitors was based on the knowledge of the catalytic mechanism of the enzyme, and X-ray structures of the murine and human enzyme, modelled with these inhibitors, suggested that the urea is the central pharmacophore. The urea pharmacophore seemed to be the most potent inhibitor, but with suitable substituents, amides and carbamates of equal potencies can be obtained. Subsequent modifications to improve in vivo stability of sEH allowed evaluation of the role of this enzyme in cardiovascular diseases. Although mEH and sEH share the same catalytic mechanism, researchers have discovered that it is possible to design inhibitors that are more than 1000 times more selective for one hydrolase over the other by using specific substituents on the amides and ureas. The sEHIs that have been tested so far are highly selective for sEH, and the more than 300 positive hits from a National Institutes of Health screen of sEHs do not consistently inhibit other enzymes. Sorafenib, an anticancer drug that is a potent inhibitor of several kinases, is also a potent sEH inhibitor. This joint inhibition seems to be limited to sEHIs that have a closely related chemical structure to sorafenib. The sEH inhibition by sorafenib may potentially reduce some of the side effects that are associated with this drug class when drugs in this class are used at high doses [24].

The first report to show in vivo biological effects of a sEHI used a single bolus dose of N, N'-dicyclohexylurea, which lowered blood pressure in hypertensive rats. Chronic administration was first achieved with 1-cyclohexyl-3-dodecyl-urea (CDu), which had antihypertensive actions when injected intraperitoneally for 4 days. However, these large-molecular-mass ureas have limited solubility in both water and organic solvents, and so careful formulation is needed to demonstrate in vivo efficacy [25].

Researchers have found that incorporating functional polar groups into one of the alkyl chains of 1,3-disubstituted urea sEHIs results in compounds that are weak structural mimics of EETs with improved physical properties. One example is 12-(3-adamantan-1-yl-ureido) dodecanoic acid (AuDA), which has been widely used in cultured cells and animals. However, AuDA requires dimethyl sulphoxide (DMSO) for in vitro experiments, and a considerable amount of 2-hydroxyl propyl β-cyclodextrin for it to be administered in drinking water for in vivo studies. If lipophilic compounds do not remain in solution, bioavailability decreases dramatically.

The addition of a polar group, called a secondary pharmacophore, such as an ether, ester, amide, sulphonamide, alcohol or ketone approximately 7–8 Å from the polar group of the central pharmacophore increases the water solubility of the inhibitor without reducing potency. This concept has been used to produce other drug-like sEHI molecules, including trans-4-[4-(3-adamantan-1-yl-ureido)-cyclohexyloxy]-benzoic acid (t-AuCb), 1-trifluoromethoxyphenyl-3-(1-acetylpiperidin-4-yl) urea (TPAu), and others that have excellent potency and efficacy in many species. Alongside the improvement of sEHIs for experimental studies, the development of sEHIs for use in humans has advanced, with sEHIs initially being developed for the treatment of hypertension [25] (As shown in fig no.2).

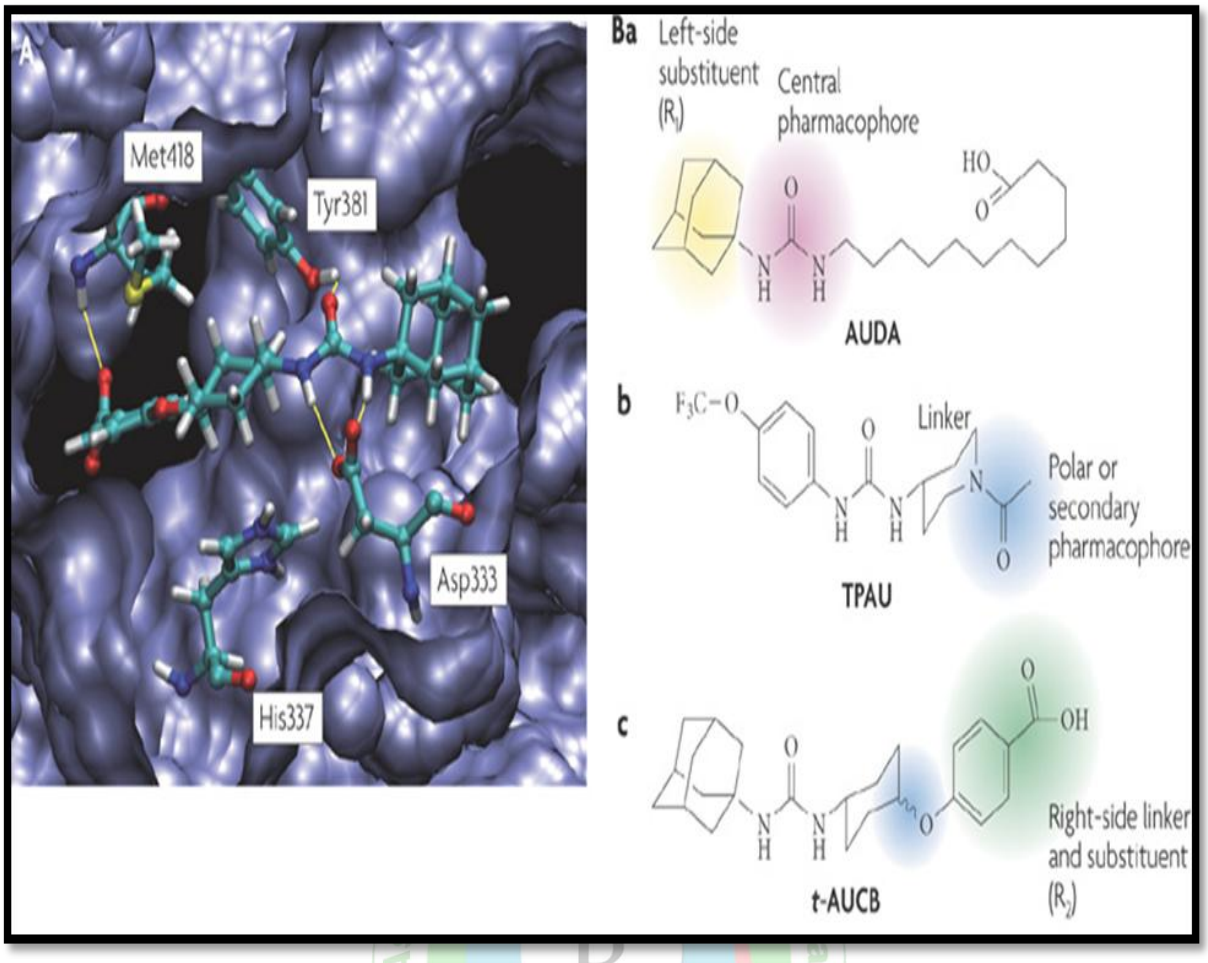

Figure No. 2: (A) This image shows the structure of sEH's enzymatic pocket with sEHI and t-AUCB bound within. Tyr465 residue is omitted for clarity. The structure was prepared with Scigress Explorer Standard version 7.7.0.49, using the atomic coordinates from the Protein Data Bank (PDB number 1VJ5). The image was produced with VMD 1.8.6 and POV-Ray 3.6 and highlights the amino-acid residues that form the sEHI binding site. (Ba) AUDA is a potent sEHI compound with a central pharmacophore that contains urea, amide, and carbamate substituents. The R1 or left side of the molecule is located in a hydrophobic pocket of the sEH catalytic tunnel while the right side is designed to mimic EET. AUDA should be carefully formulated for in vivo use. (Bb) TPAU is a potent sEHI with a polar secondary pharmacophore linked to the central pharmacophore by a piperidine group. This secondary pharmacophore is located 7-8 Å away, which increases solubility while maintaining potency. (Bc) t-AUCB and TPAU are highly potent sEHIs with good oral availability and pharmacokinetic characteristics. t-AUCB has an ether as its secondary pharmacophore, mimicking the carboxylate of EETs. Both the cis isomer and t-AUCB are active sEHIs. t-AUCB is more broadly active across multiple species. Image courtesy of S. H. Hwang, University of California, Davis.

Cardiovascular Therapeutic Effects ofsEHIs: -

sEHIs have been found to have cardiovascular-protective effects in several diseases, including hypertension, cerebral ischaemia, cardiac ischaemia, cardiac hypertrophy, and atherosclerosis. This suggests that these agents have the potential to treat many cardiovascular diseases and associated morbidity. sEH inhibition has also been shown to attenuate the progression of end-organ damage, inflammation, and endothelial dysfunction that are associated with cardiovascular disease (As shown in fig no.3). Studies conducted in mice with Ephx2 gene deficiency suggest that the effects of sEHIs are due to the inhibition of the C-terminal epoxide hydrolase domain. These studies have the potential to reveal the function of the N-terminal domain of the sEH enzyme, although a role for this domain has remained elusive [26].

Anti-Hypertensive Effect: -

Many animal models of hypertension have shown that sEHIs have antihypertensive effects, as seen in Figure. In the SHR, urea N,N'-dicyclohexylurea lowered blood pressure and decreased urinary DHET excretion, while CDU, given once daily, lowered blood pressure in rats with hypertension driven by angiotensin infusion. AuDA, the first sEHI to be successfully administered orally to hypertensive animals, lowered blood pressure in rat and mouse models of hypertension. Blood pressure was consistently lowered by 25-30 mmHg in rat models of hypertension and to a greater extent in mice that had angiotensin-dependent hypertension. The mechanism by which AuDA lowers blood pressure seems to be dependent on decreased vascular resistance and enhanced Na+ excretion by the kidney, which is consistent with the biological actions of EETs to dilate blood vessels and inhibit renal tubular Na+ reabsorption. It was also in these initial hypertension studies that the first evidence for end-organ protection by sEHIs was recognized [27].

There have been some conflicting reports on sEHI-mediated lowering of blood pressure in rats and mice, which may be due to differences between rat strains in sEH activity. Although the first demonstration of the ability of sEHIs to lower blood pressure was in the SHR, subsequent studies have shown variable levels of blood pressure-lowering in

this model, which could be due in part to polymorphisms in the Ephx2 gene between SHR strains. There are also conflicting results on sEHI-mediated changes in blood pressure in Ephx2–/– mice. The initial Ephx2–/– male mice had decreased blood pressure that could not be confirmed when these mice were back-bred into a C57/bL6 background or in an independently generated Ephx2–/– mouse colony. More recently, studies in a second Ephx2–/– C57/bL6 back-bred colony did not show lower blood pressure in males at baseline, but deoxycorticosterone (DOCA)-salt-induced hypertension was attenuated. Interestingly, each Ephx2–/– mouse colony had higher levels of EET and 20-HETE than controls, which could have offset some of the blood pressure effects. Despite variable antihypertensive actions, sEHIs have consistently shown the ability to protect from end-organ damage associated with cardiovascular diseases[28].

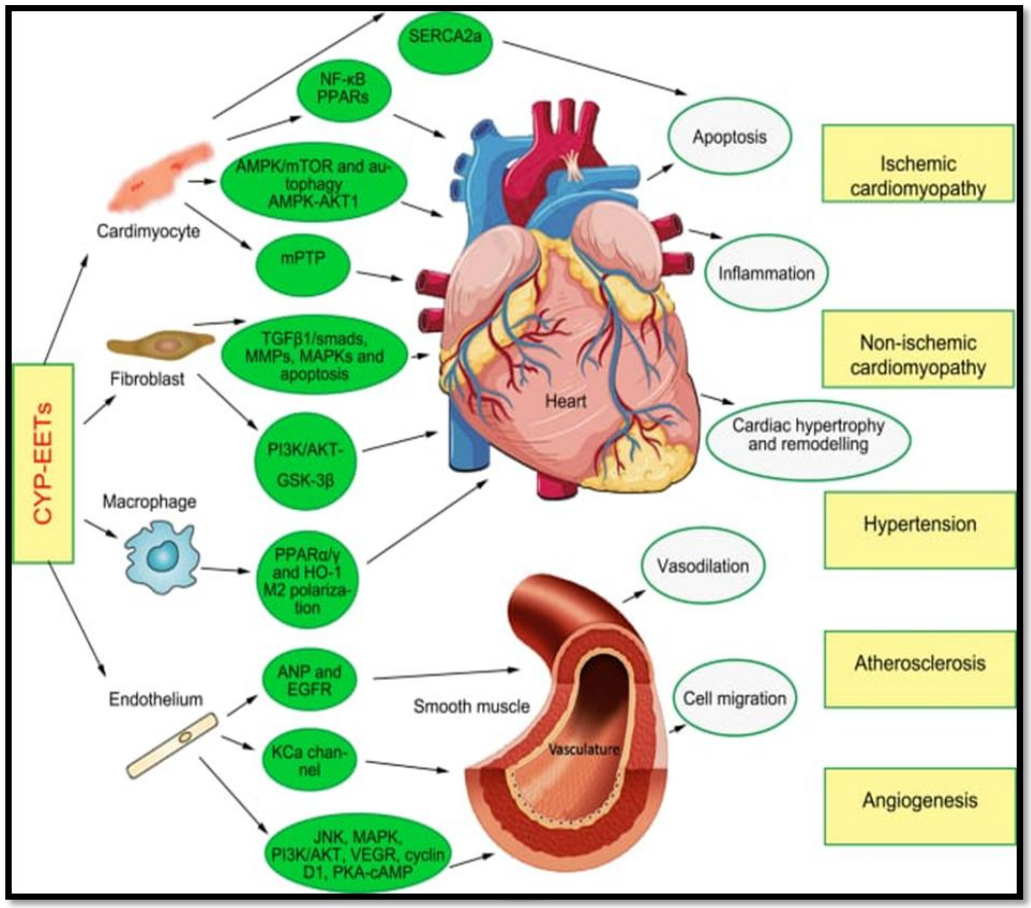

Figure No. 3: CYP epoxygenase metabolites of AA, also known as CYP-EETs, play a crucial role in the proper functioning of the cardiovascular system. CYP-EETs act as lipid mediators that trigger various biological responses and affect both vascular and cardiac functions. They have been found to have anti-apoptotic and anti-inflammatory properties, promote vasodilation, induce angiogenesis, lower blood pressure and help against ischemic and non-ischemic cardiomyopathy.

Kidney Protective Properties: -

Chronic sEHI treatment has been found to reduce renal vascular and glomerular injury in rats with angiotensin-induced hypertension. This indicates that sEHIs can protect against the end-organ damage related to cardiovascular disease. In the rat model, sEHIs decreased collagen expression in glomeruli and tubular cells, and reduced vascular hypertrophy. Additionally, sEHI treatment led to a decrease in macrophage infiltration and urinary albumin excretion. The protective effect of sEHIs on the kidneys was observed whether treatment began at the onset of hypertension or after its establishment. While the decrease in blood pressure could have contributed to this effect, a recent study in diabetic Goto-Kakizaki rats demonstrated that AuDA provides renal protection independently of lowering blood pressure. This study also found that the elevated plasma cholesterol and triglyceride levels in these rats were not affected by AuDA treatment. Studies in mice have also shown that sEHIs can protect against acute renal injury induced by the chemotherapeutic agent cisplatin. Inhibition of sEH decreased blood urea nitrogen levels for up to 96 hours and reduced the tubular damage associated with cisplatin. Overall, these studies consistently indicate that sEHIs can improve renal vascular function, decrease glomerular injury, and reduce renal inflammation. This highlights the potential of sEHIs as a treatment for acute and chronic kidney disease [29].

Cardiac Protective Properties: -

sEHIs possess cardiac-protective properties that make them a promising therapeutic option for preventing myocardium ischaemic events. Studies on Ephx2-deficient mice have shown that these mice have a better recovery of left ventricular developed pressure and reduced infarct size after ischaemia and reperfusion. Additionally, these mice are protected from developing pressure overload-induced heart failure and cardiac arrhythmias. The cardiac function improvement provided by sEHIs has been established in

various experimental models and species. AuDA has been shown to reduce the cardiac infarct size in dogs, similar to the effect observed with 14,15-EET administration. Similar effects were observed in mice administered AuDA butyl ester (AuDA-bE) and subjected to left coronary artery occlusion followed by reperfusion. Furthermore, the EET antagonist 14,15-epoxyeicosa-5(Z)-enoic acid (14,15-EEZE) inhibits the cardiac-protective effects of sEHIs in dogs and mice [26].

Cardiac hypertrophy caused by ventricular remodelling is a common outcome of acute myocardial infarction or hypertension. Studies have shown that sEHIs can attenuate cardiovascular hypertrophy. Rats with DOCA-salt-induced hypertension treated with a sEHI exhibited decreased heart weight and collagen levels. Similarly, inhibition of sEH has been shown to prevent cardiac hypertrophy in stroke-prone SHRs and angiotensin-infused rats. Studies conducted on mice with pressure overload-induced myocardial hypertrophy have shown that sEHIs prevented or reversed the development of left ventricular hypertrophy. This effect was linked to the ability of sEHIs to block NF-κb activation [30].

While there is an abundance of evidence supporting the cardiac-protective effects of Ephx2 deficiency and sEHIs, Ephx2-knockout mice exhibited reduced survival from cardiac arrest during cardiopulmonary resuscitation. More experimental evidence is required to determine the potential of sEHIs as a therapy for various heart diseases [26,30].

Protection against ischaemic stroke and vascular disease:

sEHIs have potential therapeutic use in preventing ischaemic brain damage caused by stroke. Chronic treatment with AuDA in hypertensive rats decreases cerebral infarct size after middle cerebral artery occlusion. Interestingly, blood pressure is not affected, which supports the idea that cerebral-protective effects are independent of blood pressure. Ephx2-deficient mice also have decreased infarct size following cerebral ischaemia. In a mouse model of focal ischaemia–reperfusion injury, administration of AuDA-bE or exogenous EETs resulted in at least a 50% reduction in infarct volume. Moreover, administering sEHIs 1 hour before or at the start of reperfusion provided cerebral protection. The mechanisms by which sEHIs protect the brain from ischaemic damage involve the cerebral vasculature and neurons. EETs and sEHIs can protect neurons through anti-apoptotic and anti-inflammatory actions, and vasodilatory EETs regulate cerebral blood flow, which could contribute to brain protection (As shown in fig no. 3). Angiogenic and attenuated vascular remodelling that allow for enhanced perfusion of the ischaemic area have been observed in hypertensive rats treated with sEHIs. However, these vascular changes do not occur in normotensive animals that also demonstrate decreased infarct volume when treated with sEHIs. These findings indicate that sEHIs have broad pharmacological potential for treating ischaemic stroke. Other areas that are beginning to be explored include the effects of sEHIs on vascular remodelling, angiogenesis, diabetes and atherosclerosis. In rats and cultured human cells, inhibition of sEH decreased vascular hypertrophy in hypertension and decreased vascular smooth muscle cell proliferation. In mice, angiogenic actions of EETs have been shown that were enhanced in the presence of a sEHI. Increased microvascular densities and increased middle cerebral artery compliance were associated with AuDA treatment in hypertensive rats. Recent studies have shown that sEHIs or Ephx2 deletion antagonizes neointimal formation in vivo by mechanisms that are endothelium-dependent. Atherosclerosis in Apo lipoprotein E-knockout mice was also reduced by sEHI treatment. Therefore, sEHIs may have therapeutic potential for specific types of vascular remodelling and atherosclerosis [31].

Anti-Inflammatory Properties: -

Inflammation plays a key role in cardiovascular and other diseases. sEHIs, or soluble epoxide hydrolase inhibitors, have been found to have anti-inflammatory properties that make them effective in protecting organs affected by such diseases. Moreover, they can also be used to treat inflammatory diseases [32]. Studies have shown that sEHIs reduce the production of cytokines and pro-inflammatory lipid mediators, and prevent lipopolysaccharide-induced mortality in mice. In rats, topical application of sEHIs has been found to reduce lipopolysaccharide-induced thermal hyperalgesia and mechanical allodynia, both of which are symptoms of inflammatory pain. However, sEHIs have not been found to reduce lipopolysaccharide-induced expression of inflammatory genes or neutrophil accumulation in the liver in mice deficient in Ephx2 or wild-type mice that were administered a sEHI. Nevertheless, sEHIs have therapeutic value in reducing lung inflammation caused by tobacco smoke in mice, as they reduce macrophage infiltration into the rat lung exposed to tobacco smoke. Overall, sEHIs have beneficial anti-inflammatory and analgesic effects [33].

Epoxyeicosanoids as a therapeutic target: -

The angiogenic properties of sEHIs and EETs suggest that inhibiting epoxygenase enzymes or EETs could be a potential treatment for tumour growth. It is worth noting that the CYP2J2 epoxygenase enzyme is often upregulated in many tumours [34]. Recent studies have revealed that CYP2J2 inhibitors that decrease EET production can significantly suppress tumour growth in both in vitro and in vivo settings, including various human cancer cells. EET antagonists such as 14, 15-EEZE or sEH protein may also have potential as cancer therapeutics. Epoxyeicosanoids and EET analogues are being investigated as potential therapeutic agents for cardiovascular diseases [35]. Increasing EET levels or overexpressing epoxygenase enzymes have shown to have cardio protective effects. The identification of binding sites or receptors for EETs could aid in discovering new targets for the treatment of cardiovascular diseases. Recent evidence suggests that EET analogues can be designed for chronic administration to SHRs and have antihypertensive actions. A combination drug with EET mimetic actions and sEHI activity is also a possible approach. EET analogues might also be useful for treating acute myocardial infarction and improving the effectiveness of drug-eluting stents [35].

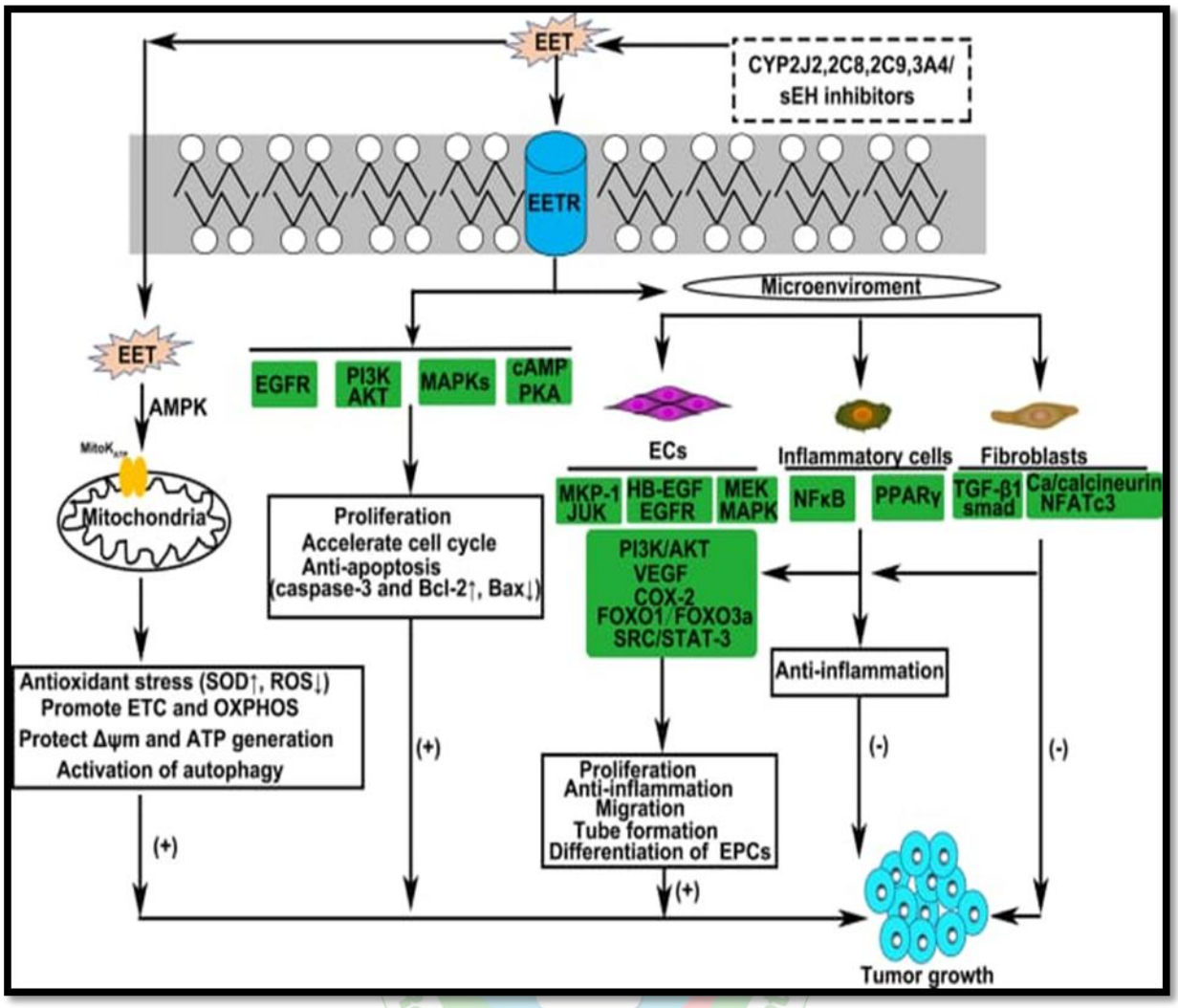

Figure No. 4: EETs enhance tumour growth by accelerating proliferation, cell cycle, and protecting carcinoma cells from apoptosis through multiple pathways. They also improve mitochondrial function, regulate cells in the tumour microenvironment, and prevent oxidative stress damage.

CONCLUSION:

Since the first description of their antihypertensive actions in 2000, significant progress has been made in evaluating sEHIs as a therapy for cardiovascular diseases. However, future research is required to explore other non-cardiovascular diseases that could potentially be treated with sEHIs. The evidence suggests that sEHI treatment may benefit inflammatory diseases, neurological diseases such as Alzheimer's disease, and diseases associated with pain. Therefore, the sEHIs have great potential in the treatment of cardiovascular diseases, and other potential therapeutic applications seem to be on the horizon.

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