Soluble Epoxide Hydrolase Inhibitors: New Molecules with Potential for Use in Veterinary Medicine

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ABSTRACT

Treatments for inflammation and pain are important consideration in human and veterinary medicine. The classical drugs for treatments of inflammation and pain act by inhibition of cyclooxygenase and lypoxygenase pathways. However, there is still a need to develop new veterinary drugs and trials to apply human drugs to the veterinary field in order to increase the veterinary drug armamentarium. However, it is pivotal to experimentally test human drugs and therapies in veterinary species before veterinary clinical applications. The soluble epoxide hydrolase inhibitors (sEHIs), are novel active ingredients shown to have a number of beneficial effects. This has been especially demonstrated in many animal models in relation to inflammation and pain. The present review reports the state of the art of soluble epoxide hydrolase inhibitors and suggests their potential use in veterinary medicine.

Keywords: Soluble epoxide hydrolase, epoxyeicosatrienoic acids, inflammation, pain.

INTRODUCTION

Pets are treated as members of the family and pet owners demand the same level of care they expect for themselves. This change in attitude has led to a rapid evolution in the field of pharmacology with a trend towards the development of more effective and innovative veterinary therapies with higher potency, more rapid speed of action and fewer side effects (1).

Treatment of pain and inflammation are important considerations in human medicine. Likewise, in veterinary medicine in recent years, pain has been shown to dramatically affect animal welfare and production, and interest in the field of analgesia is increasing (2). Furthermore, veterinary pharmacology still has a limited drug armamentarium and human drugs are increasingly being investigated for veterinary use. It has only been in recent years that analgesics have been marketed exclusively for veterinary patients. Therefore, it is pivotal that new human drugs and therapies be tested also in veterinary species (1).

The two main classes of drugs used to reduce pain in animals are opioids and nonsteriodal anti-inflammatory drugs (NSAIDs). Recently, some of the novel molecules in these classes marketed for the human field have been successfully tested on veterinary species (3-5). In the last few years, many researchers have directed attention towards arachidonic acid (AA) metabolism and in particular, to the cytochrome P450 (CYP450) enzymes. These have been referred to as the third pathway of AA metabolism, in addition to cyclooxygenases (COX) and lipoxygenases (LOX) (6).

All AA metabolites, which encompass the prostanoids, leukotrienes and epoxy fatty acids (Figure 1), are bioactive lipids that play a positive or negative role in inflammation and pain, specifically under pathological conditions. The allogeneic and pro-inflammatory prostanoids and leukotrienes drive and maintain inflammation, while the anti-inflammatory and analgesic epoxy fatty acids epoxyeicosatrienoic acids (EETs) reduce and resolve inflammation (7).

Compared to the well-recognized products of the COX and LOX branches of the AA cascade, the EETs generated by CYPs were only discovered in the early 1980s (7). EETs have various beneficial biological effects: not only do they have anti-inflammatory and analgesic actions but they also have protective effects on the cardiovascular system and kidney as recently reported in the literature (8-19) (Table 1).

Table 1: Functions of EETs

Functions	References
Effect on sodium ion channel	8
Increases in intracellular Ca^{2+}	9
Activation of K_{ATP} ion channel	10
Ca^{2+} signalling	11, 12
Activation of BK_{Ca} ion channel	13
Myocardiocyte contraction	14
Effect on heart ischemia	15
Inhibition of PGE2	16
Inhibition of IκB kinase (IKK)	17
Mitogenesis	18
Fibrinolysis	19

EETs are metabolized by various pathways, however the main pathway of their metabolism is through conversion to the corresponding 1,2 diols (dihydroxyeicosatrienoates, DHETs), has a less bioactive molecular structure that is characterized by a pro-inflammatory action.

The enzyme that carries out this reaction is the soluble epoxide hydrolase (sEH), and its inhibition could stabilize EET levels with expected beneficial biological effects (Figure 1).

The purposes of this review are: 1) to report the current status of preclinical studies on drugs inhibiting the soluble epoxide hydrolases (sEH) enzyme; 2) to evaluate the use of these novel active ingredients in veterinary medicine so that they can be used in the near future, thus increasing the veterinary drug inventory.

ARACHIDONIC ACID CASCADE AND CYP450 PATHWAY

Figure 1 shows the arachidonic acid cascade and its metabolism to eicosanoid mediators via three pathways, namely the COX, LOX and CYP450 pathways. CYP enzymes, which are mainly expressed in the liver, gut and kidney, are responsible for the metabolism of xenobiotics and many pharmaceuticals, but they also utilize endogenous compounds as substrates, such as cholesterol and fatty acids (6). Arachidonic acid, as shown the Figure 2, is not the only endogenous CYP substrate. CYP enzymes are also able to generate epoxides from n-6 fatty acids, linoleic acid and n-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Biological activity has been attributed to almost all of these CYP derivatives, however, the specific enzymes involved in the conversion of linoleic acid, EPA, and DHA are less well studied than those that metabolize AA (6). Via unique mechanisms, CYP450 metabolises EETs by incorporating them into phospholipids, chain shortening, chain elongation, hydroxylation and other pathways, however, the dominant pathway is the hydration of the epoxides to the corresponding 1,2-diols by soluble epoxide hydrolases (sEH) (20). sEH is a member of the epoxide hydrolases class which, in turn belongs to a sub-category of a broad group of hydrolytic enzymes that include esterases, proteases, dehalogenases and lipases (21).

In mammalian species, there are at least five epoxide hydrolase forms characterized by two different domains: N-terminal and C-terminal. The biological role of these domains is not yet well known but the C-terminal domain is an α/β hydrolase fold structure and is responsible for the epoxide hydrolase activity that catalyzes the hydration of chemically reactive epoxides to their corresponding diol products (21).

In conclusion, EETs have an important role especially in vascular, renal, and cardiac systems and modulated gene expression. They also induce vasorelaxation which likewise, has anti-inflammatory effects (22), but they are quickly metabolize by the sEH enzyme into corresponding less bioactive diols (20), reducing the beneficial effects of EETs. Therefore, the addition of sEH could be an efficient way to increase EET levels and to maintain their beneficial effects.

THE BIOLOGICAL EFFECTS OF SEHIS

As mentioned above, increasing EETs by sEHIs maintains their beneficial autocrine and paracrine effects. A number of studies concerning sEHIs have been carried out in different animal models (Table 2) to confirm this effect. sEHIs could be useful in the treatment of hypertension, atherosclerosis, pulmonary diseases, inflammation, and pain. However, the most studied and attractive targets are related to the treatment of inflammation, pain and cardiovascular diseases.

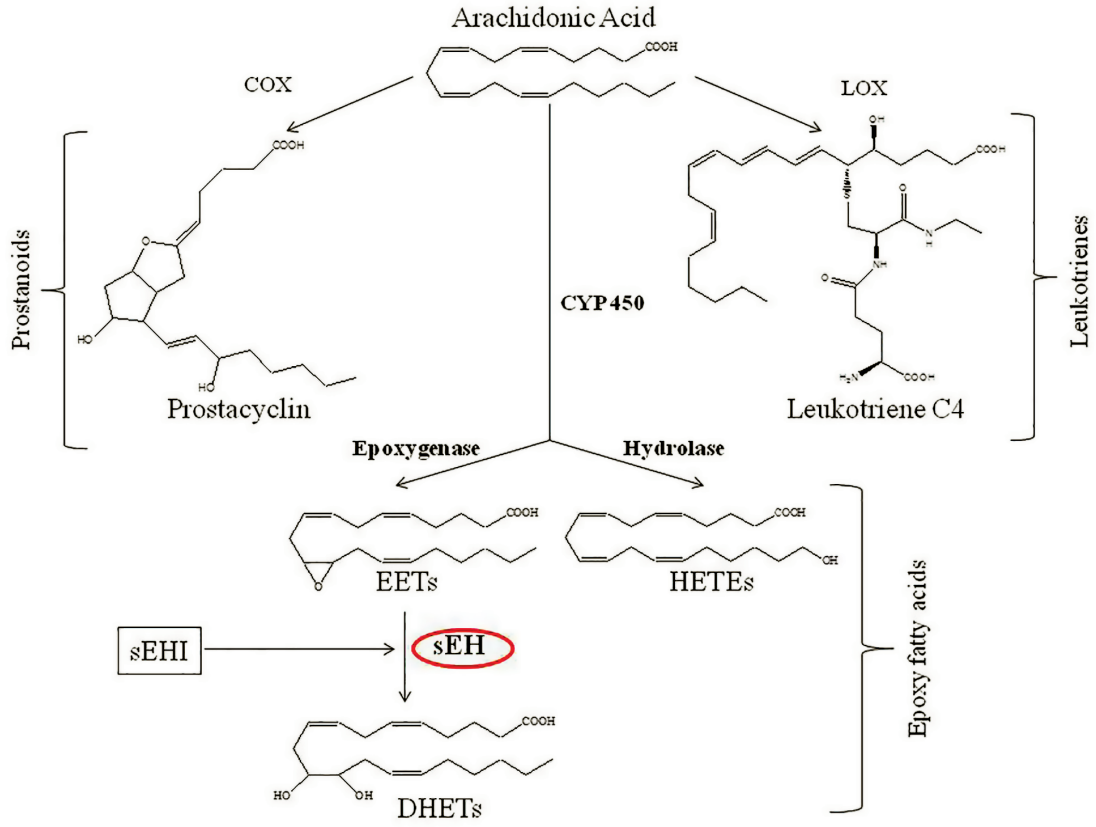

Figure 1: Arachidonic acid cascade and its major pathways metabolism. Arachidonic acid is metabolized by cyclooxygenase (COX) and lipoxygenase (LOX) enzymes into predominantly pro-inflammatory metabolites which are prostanoids and leukotrienes, respectively. The third pathway involves the cytochrome P450 enzymes that metabolize arachidonic acid into anti-inflammatory metabolites epoxy eicosatrienoic acid (EETs). EETs are rapidly metabolized by the epoxide hydrolase (sEH) to their corresponding diols dihydroxyeicosatrienoic acids (DHETs). sEH inhibitors (sEHI) block this degradation and stabilize EET levels while reducing DHETs.

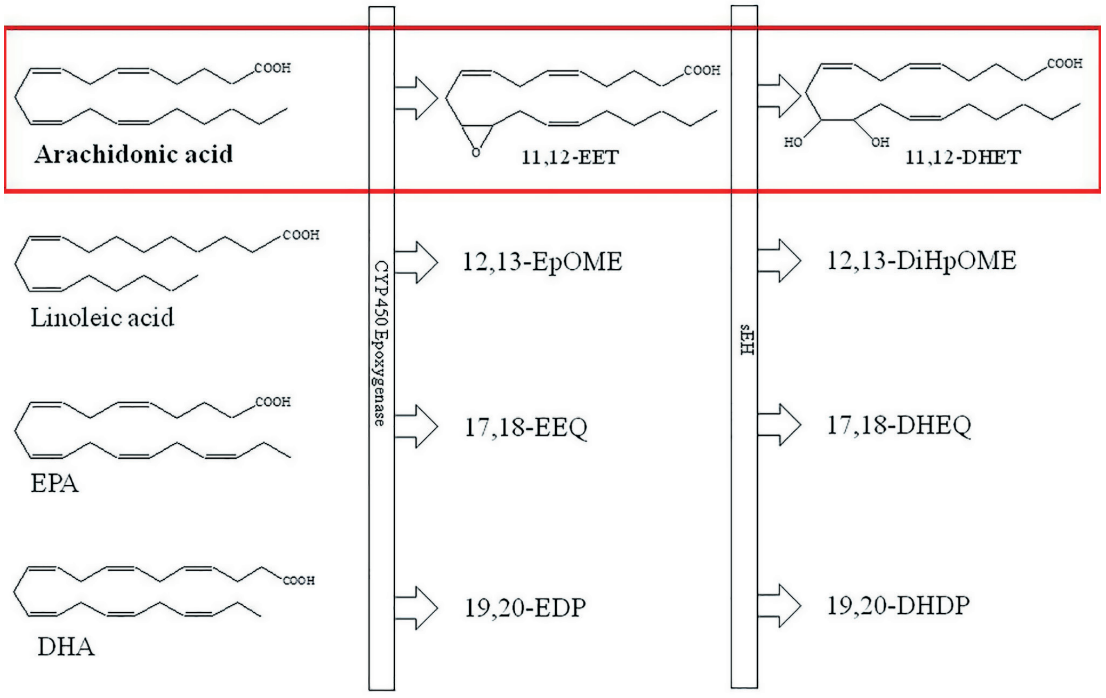

Figure 2: Endogenous CYP substrate arachidonic acid, linoleic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), their epoxydation by converting into epoxyeicosatrienoic acid (EET), epoxyoctadecenoic acid (EpOME), epoxyeicosatetraenoic acid (EEQ), epoxydocosapentaenoic acid (EDP), and metabolism of epoxides generated to the corresponding diols, by the epoxide hydrolase (sEH), dihidroxyeicosatrienoic acid (DHET), dihydroxyoctadecenoic acid (DiHOME), dihidroxyeicosatetraenoic acid (DHEQ) and dihydroxydocosapentaenoic acid (DHDP).

Table 2: Various pharmacological effects of sEHIs

Pharmacological Effects	Species	References
Anti-hypertensive effect	Mouse	26
	Rat	27, 28
Myocardial protective effect	Mouse	46, 47
Anti-atherosclerosis effect	Mouse	48
Pulmonary vasoconstrictive effect	Mouse	33
Renal vasodilatory effect	Rat	49
Anti-inflammatory effect	Mouse	35, 38
	Rat	39, 43
Analgesic effect	Rat	43, 50
	Horse	45

Several sEHIs have been widely used and evaluated for their biological effects in animal models. The first sEHIs discovered were substituted chalcone oxides: these demonstrated low efficacies *in vivo* (23). When the newer urea- and carbamate-based compounds were discovered, they showed a better metabolic stability and safety profile than their predecessors (24). However, none of these compounds have been launched on the market as yet, as a thorough evaluation of their therapeutic effectiveness and safety profile is lacking.

Cardiovascular effects

Vasodilation is a one of the major biological effects of the EETs (25). A number of investigators have demonstrated that sEHIs could be used to improve hypertension. These active ingredients have been shown to reduce hypertension in many animal models, with an efficacy similar to angiotensin II (26), deoxycorticosterone (27), salt and high fat diet (28). It has been reported that sEHIs reduce pulmonary vascular remodeling and delay pulmonary hypertension in monocrotaline induced pulmonary hypertension in rats (25). Imig *et al.* (29) demonstrated that the sEHI drug NCND (N-cyclohexyl-N-dodecyl urea) reduced arterial blood pressure in angiotensin II hypertensive animals. Moreover, other works showed protective effects of sEHIs against cardiovascular diseases. It has been reported that EETs reduce adverse effects of stress on mitochondrial potassium channels (30). Wang *et al.* (31) suggested that sEHIs showed a potential therapeutic effect in the treatment of atherosclerosis. The reduction of low-density lipoprotein and elevation of high density lipoprotein cholesterols were correlated with the anti-atherosclerotic effects of sEHI. In wild-type mice, sEHI (AUDA-BE, (12-(3-adamantan-1-yl-ureido)-dodecanoic acid butyl ester)) reduced infarct size after regional myocardial ischemia-reperfusion injury *in vivo* (32) while Xu *et al.* (33), showed that sEHIs reversed cardiac hypertrophy using a murine model of pressure induced cardiac hypertrophy.

Anti-inflammatory effect

sEHIs appear to exert their anti-inflammatory effect through stabilization of EET levels. A number of investigators have demonstrated that EETs reduce inflammation. Node *et al.* (17) demonstrated that physiological concentrations of EETs or overexpression of CYP2J2 decreased cytokine-induced endothelial cell adhesion molecule expression, and EETs prevented leukocyte adhesion to the vascular wall by a mechanism involving inhibition of transcription factor NF-κB and IκB kinase (IKK). NF-κB plays a key role in cytokine mediated inflammation and could be inactive while bound to IκB. Thus, sEHIs indirectly maintain NF-κB in the inactive state correlated with inhibition of IKK. Furthermore, inhibition of sEH enzymes led to increased anti-inflammatory properties related to regulation of cytokines (17).

Several studies have evaluated the anti-inflammatory effect of sEHIs in different inflammatory disease models. The endotoxin-induced model is a common form of the septicemic model (34); lipopolysaccharide (LPS), also known as endotoxin, is the primary Gram-negative bacteria surface antigen which causes a number of pathophysiological changes associated with eliciting immunologic responses including leukocyte activation, cytokine production and enhanced pro-inflammatory gene expression. In the LPS induced inflammation model in mice, the sEHI (AUDA-BE, (2-(3-adamantan-1-yl-ureido)-dodecanoic acid butyl ester)) decreased the production of nitric oxide metabolites and pro-inflammatory cytokines and prevented mortality (35). In another study, the sEHI (t-AUCB) significantly reduced plasma levels of pro-inflammatory cytokines such as TNF-α and IL-6 at 24 h after treatment in the LPS-treated murine model (36).

As mentioned above, EETs are converted to DHETs corresponding diols, and the blockage of this conversion by sEHIs has an important role in reducing inflammation. LPS, in particular increases the conversion into diols, decreasing the ratio of epoxides to diols. Blood epoxide and diol levels in normal animals treated with sEHIs are lower than those in inflammatory animals (37). This was clearly shown

by Liu *et al*. (38) in LPS-treated mice where sEHIs such as AUDA-BE, significantly reduced the production of diols and increased the ratio of epoxides to diols. Thus, sEHIs have been shown to have therapeutic efficacy in the treatment of endotoxin induced inflammation.

Moreover, in tobacco smoke-exposed rats, the sEHIs (AUDA-nBE, AUDA n-butyl ester) facilitated a decrease in bronchoalveolar inflammatory cells, including significant reductions in alveolar macrophages, neutrophils, and lymphocytes (39). Additionally, co-administration with EETs further reduced the number of bronchoalveolar inflammatory cells (39).

In summary, the anti-inflammatory effects of sEHIs have been shown to result through multiple pathways. sEHIs reduce the production of cytokines and pro-inflammatory lipid mediators. Stabilizing EETs by sEHIs led to down regulation of other enzymes such as COX-2 and 5-LOX in the AA cascade. In addition, the co-administration of NSAIDs and sEHIs produced an antinociceptive effect in an inflammatory pain model. Indeed a synergistic action in reducing predominantly inflammatory eicosanoids like prostaglandin PGE2 has been shown (37). Hence, it was speculated that COX inhibitors can increase EET levels and that stabilized EETs can improve anti-inflammatory effects (40). This is in agreement with results from the inflammatory rat model, where co-administration of a sEHI with a low dose of celecoxib (a COX-2 selective inhibitor) was highly effective against inflammation (41). Moreover, co-administration of sEHIs and COX inhibitors reduced the side effects of the COX inhibitor, improving their safety. Thus, the sEHIs should allow reducing the dose of COX inhibitors required for the treatment of inflammation.

Inflammatory pain

EETs dramatically reduce PGE2 levels, a cytokine with a central role in inflammation and pain, therefore, sEHIs could be used in cases of inflammatory pain to reduce production of painful mediators of inflammation. This is in line with the analgesic effect showed in animal models.

sEHI showed similar efficacy and a 1000 fold increase in potency compared to morphine in inflammatory pain models (42). According to Inceoglu *et al* (43), topical application of sEHIs effectively attenuates thermal hyperalgesia and mechanical allodynia in LPS-treated rats. Moreover, co-administration of EETs with a sEHI showed an additive increase in anti-hyperalgesia and sEHIs were demonstrated as acting in both peripheral and central nerves systems (44).

Veterinary applications

In the field of veterinary medicine, the first application of sEHI was conducted in 2013. This study tested if the sEHI (t-TUCB, trans-4-{4-[3-(4-trifluoromethoxy- phenyl)-ureido]-cyclohexyloxy}-benzoic acid) might reduce severe inflammatory pain in a horse affected by laminitis (45). The patient in this study was a horse treated for laminitis for a 7-day period using different NSAIDs and gabapentin. Treatments with these classical drugs were not effective and euthanasia was being considered for humane reasons. On day 8, a sEHI was added to the treatment protocol. After the first dose, the horse began to walk spontaneously, had a good appetite with remarkable reduction in pain scores and no side effects were reported (45).

In conclusion, the studies presented in this review are quite persuasive in demonstrating that sEHIs may have potential applications in the treatment of several diseases. Moreover, a recent study has also demonstrated effectiveness of these drugs in veterinary medicine (45). Hopefully, this is the first of many sets of data to be generated regarding the successful treatment of pain in animals. These new active ingredients could be particularly applicable in animals sensitive to the common anti-inflammatory drugs.

CONFLICT OF INTERESTS

None of the authors has any financial or personal relationship that could inappropriately influence the content of the paper.

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