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Use of Metabolomic Profiling in the Study of Arachidonic Acid Metabolism in Cardiovascular Disease

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Abstract

Arachidonic acid is one of the pivotal signaling molecules associated with inflammation, pain and homeostatic function. Drugs specifically targeting these signaling pathways represent more than 25% of annual pharmaceutical sales worldwide. However, chronic administration of nonsteroidal anti-inflammatory drugs (NSAIDs) and rofecoxib (Vioxx), a potent cyclooxygenase-2 inhibitor, have been associated with adverse cardiovascular events. Understanding the possible mechanisms underlying these adverse events is critical for evaluating the risks and benefits of this group of drugs and for development of safer drugs. Using a powerful metabolomics approach, 20-hydroxyeicosatetraenoic acid (20-HETE) was identified among many of arachidonic acid metabolic products as a likely culprit for adverse cardiovascular side effect associated with rofecoxib and NSAIDs. In addition, using a similar metabolomic approach, epoxyeicosatrienoic acids (EETs), which are lipid mediators derived from arachidonic acid through the cytochrome P450 epoxygenase pathway, have been shown to exhibit cardioprotective effects in a murine myocardial infarction (MI) model. Inhibitors of the soluble epoxide hydrolase increase titers of epoxy fatty acids and both block and reverse cardiac hypertrophy in rodent models. These highly potent, orally available compounds may be promising for treating heart failure and other cardiovascular disease. In this review, we will summarize some of the recent advances using metabolomic profiling to gain insights into the involvement of arachidonic acid pathways in cardiovascular disease.

Introduction

Arachidonic acid is a polyunsaturated omega-6 fatty acid which is released in response to tissue injury. Arachidonic acid represents one of the pivotal signaling molecules involved in the initiation and propagation of diverse signaling cascades regulating inflammation, pain and homeostatic function. Drugs developed to target these signaling pathways represent more than 25% of annual pharmaceutical sales worldwide. Arachidonic acid is metabolized through three enzymatic pathways. The cyclooxygenase (COX) pathway produces prostanoids. The lipoxygenase (LOX) pathway yields monohydroxy compounds and leukotrienes, while the cytochrome P450 (CYP450) epoxygenase pathway generates hydroxy and epoxyeicosanoids. This group of lipid mediators, which are derived from the 20-carbon atom arachidonic acid or similar fatty acids, is collectively referred to as eicosanoids (“eicosa” means 20 in Greek). A schematic metabolic pathway of arachidonic

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acid is shown in Figure 1. There is mounting evidence that some of these metabolic products play critical roles in cardiovascular disease.

Cardiovascular disease remains one of the leading causes of death in the Western societies [1]. Cardiac failure is the final consequence of a variety of etiologies including coronary heart disease, myocardial infarction (MI), hypertension, arrhythmia, viral myocarditis, and genetic cardiomyopathies. Once heart failure develops, the condition is for the most part irreversible. Although considerable progress has been made in the pharmacologic and device management of heart failure in recent decades, the mortality in heart failure patients remains significant. Moreover, the incidence and prevalence of cardiac failure are increasing as the population ages [2].

Recently, our laboratories have taken advantage of a new technique of metabolomic profiling using liquid chromatography-tandem mass spectrometry (LC-MS/MS) to elucidate the contribution of arachidonic acid metabolism in cardiovascular diseases. Metabolomics is a promising approach that has been widely used as a powerful tool in disease diagnosis [3], biomarker discovery [4], toxicity evaluation [5], gene function [6], and pharmacological research [7, 8]. In this review, we will provide examples of the use of metabolomic profiling in our two recent studies. Liu *et al* used a broad metabolomics approach to quantify the representative oxylipin mediators derived from arachidonic and linoleic acids mediated by COXs, LOXs, and CYP450s [9]. Oxylipins are oxygenated lipids and one of the most biologically important groups of oxylipins is the eicosanoid family. Specifically, Liu *et al* applied metabolomic profiling in a murine model and identified a link between the administration of rofecoxib (Vioxx) and adverse cardiovascular events. They found a significant increase in 20-hydroxyeicosatetraenoic acid (20-HETE), a potent vasoconstrictor and the culprit for increasing risk for MI and stroke. This mechanism may be shared among other non-aspirin non-steroidal anti-inflammatory drugs (NSAIDs). In the second instance, Li *et al*, using a similar approach, demonstrated the beneficial effects of increasing epoxyeicosatrienoic acid (EETs) levels and the EETs/dihydroxyeicosatrienoic acids (DHETs) ratio by application of soluble epoxide hydrolase (sEH) inhibitors in a murine MI model.

20-Hydroxyeicosatetraenoic acid (20-HETE)

Rofecoxib is a potent, orally active, and selective COX-2 inhibitor and was previously approved by the US Food and Drug Administration to treat a wide variety of pain ranging from arthritis, dysmenorrhea, and migraine. However, rofecoxib was voluntarily withdrawn from the worldwide market in 2004 because it was found to be associated with a higher risk for adverse cardiovascular events and stroke in arthritic patients compared with those on the control naproxen [10, 11]. This resulted in lawsuits involving an almost $5 billion settlement [12]. In addition, high doses of other coxibs such as valdecoxib and celecoxib are also associated with adverse cardiovascular events.

The current theory on the possible mechanisms responsible for the observed adverse effects of rofecoxib is that rofecoxib reduces the production of prostacyclin I2, an inhibitor of platelet aggregation (PGI2 see Figure 1). This results in an increase in platelet aggregation and may predispose patients for adverse cardiovascular events including MI or stroke. On the other hand, it has long been known that NSAIDs inhibit the production of the potent platelet activator thromboxane (TX) A2 [13, 14], so these agents may have thrombolytic activities. Hence, one might expect that conventional NSAIDs are “neutral” or even beneficial to the cardiovascular system. However, a significantly increased risk for cardiovascular diseases such as MI, hypertension, and heart failure has been observed to be associated with the administration of some of the non-aspirin NSAIDs, including but not

limited to diclofenac, ibuprofen, naproxen, and indomethacin [15]. Thus, current hypothesis provides an incomplete explanation for the observed adverse cardiovascular events associated with the use of NSAIDs. We reason that this may result from the fact that the dominant theory is based on monitoring only a few arachidonate metabolites. To evaluate the risks and benefits of selective COX-2 inhibitors and to develop safe coxibs or adjuvants to improve the safety of existing coxibs, it is critical to understand the possible interactions among different arachidonic acid metabolic pathways.

We utilized a murine model which was administered with rofecoxib for a period of 3 months. In this model, there was a dramatic decrease in bleeding time which reflected an increase in platelet aggregability. Increased platelet aggregability has been associated with the pathogenesis of MI and stroke [16–19]. The quantitative levels of 27 oxylipin mediators of the plasma from treated animals were determined using metabolomic profiling. There was a greater than 120-fold increase in the plasma concentration of 20-HETE in the mice treated with rofecoxib. Moreover, a direct infusion of 20-HETE in mice also resulted in shortened bleeding time. Taken together, our data may provide a link between the use of rofecoxib and related compounds and the reported adverse cardiovascular events. This hypothesis suggests 20-HETE as a biomarker for cardiovascular risk from coxibs as well as possible strategies for attenuation of their adverse effects. For example, we predict that inhibition or down-regulation of CYP4A and or CYP4F may ablate the cardiovascular events of coxibs. In addition, this study exemplifies the use of metabolomic profiling as a promising tool to gain a more comprehensive understanding of biological processes.

Epoxyeicosatrienoic acids (EETs)

The CYP450 epoxygenase products, the epoxyeicosanoids, also known as EETs, are major anti-inflammatory arachidonic acid metabolites with a variety of biological effects [20]. There is growing evidence supporting the notion that EETs and other epoxy and diol fatty acids play a significant protective role in the cardiovascular system. EETs have been identified as potential endothelium-derived hyperpolarizing factors (EDHFs) [21, 22]. Major roles of EETs include modulation of both blood pressure and inflammatory signaling cascades. EETs are also associated with a number of other physiological functions including modulation of ion channel activity, angiogenesis, cell proliferation, vascular smooth muscle cell migration, leukocyte adhesion, platelet aggregation and thrombolysis, and neurohormone release [23, 24]. It has been proposed that diminished production or concentration of EETs contributes to cardiovascular disorders [25]. A polymorphism of the human *CYP2J2* gene, which is highly expressed in heart and active in the biosynthesis of EETs, encodes variants with reduced catalytic activity and is independently associated with an increased risk of coronary artery disease [26]. Transgenic mice with cardiomyocyte-specific over-expression of human *CYP2J2* demonstrate enhanced post-ischemic functional recovery [27] and significant protection against doxorubicin-induced cardiotoxicity [28]. As the protective role of EETs in cardiovascular biology has been increasingly recognized, considerable interest has arisen in developing methods to enhance the bioavailability of these compounds.

There are a variety of pathways involved in the degradation of EETs, but the major pathway is catalyzed by the enzyme soluble epoxide hydrolase (sEH). sEH converts EETs to their corresponding diols, dihydroxyeicosatrienoic acids (DHETs), thus modifying the function of these oxylipins [29]. Over the last few years, sEH has gained considerable attention as a therapeutic target for cardiovascular diseases [30–33]. Pharmacological inhibition of sEH has emerged as an intriguing approach to enhance the bioavailability of EETs and EET-mediated cardiovascular protective effects [29, 34–42]. The beneficial effects of several potent, orally available sEH inhibitors (sEHIs) in the prevention and reversal of cardiac

remodeling due to maladaptive hypertrophy and myocardial ischemia/reperfusion have been demonstrated in several studies, including those from our laboratory [37, 40, 43, 44].

Specifically, we tested the effects of sEHIs on prevention and reversal of cardiac hypertrophy and post-ischemia remodeling, which are among the most common causes leading to heat failure. We demonstrated that sEHIs can prevent the development of pressure-induced cardiac hypertrophy using a murine model of thoracic aortic constriction (TAC) [43]. In addition, sEHIs reversed the pre-established cardiac hypertrophy caused by chronic pressure overload, in which a high level of expression of sEH in mouse atrial and ventricular myocytes was documented [43]. More recently, our laboratory has also demonstrated the beneficial effects of sEHIs on the progression of cardiac remodeling using a clinically relevant murine model of MI [44].

Using LC-MS/MS based techniques, we documented a significant decrease in the EETs/DHETs ratio in a MI model, indicating increased sEH activity, which may play a role in the progression of post-ischemia remodeling [44]. Treatment with sEHIs resulted in the normalization of the EETs/DHETs ratio and a reduction in post-ischemia LV remodeling [44]. Moreover, we have documented that the significant decrease in the EETs/DHETs ratio in the MI model showed a striking parallel with the changes in inflammatory cytokines at 3 weeks post MI, which indicated a heightened inflammatory state [44]. Additionally, the normalization of the EET/DHET ratios by sEHIs results in a reversal of the elevated cytokine levels in the MI model. Persistent inflammation, involving increased levels of inflammatory cytokines, plays a potential pathogenic role in the progression of LV dysfunction and remodeling in heart failure [45, 46]. The sEHIs appear to change the pattern of inflammatory mediators from a state which promotes the propagation of inflammation toward one promoting resolution.

sEH has been shown to be expressed in cardiomyocytes [37]. The expression of sEH is upregulated by angiotensin II in cardiac myocytes *in vitro* and *in vivo*, suggesting a potential regulatory role of sEH in angiotensin II-induced maladaptive hypertrophy [35]. Finally, recent human epidemiological studies have identified associations between variations in EET metabolic pathway genes and increased cardiovascular risk. A polymorphism leading to reduced gene activity of *CYP2J2* is associated with an increased risk of coronary artery disease [26], and *EPHX2* has also been identified as a susceptibility factor for heart failure [47]. Taken together, these findings suggest that increased sEH activity and reduced bioavailability of EETs may play a significant role in the pathogenesis of heart failure.

Interestingly, sEHIs have been shown to indirectly down regulate the expression of COX-2 and synergize with NSAIDs towards the reduction of inflammation [48, 49]. This suggests that these drug combinations (NSAIDs and sEHIs) may produce a beneficial anti-inflammatory effect while reducing the required dose of COX-2 inhibitors, thus avoiding the adverse cardiovascular side effects attributed to COX-2 inhibitors.

Future Directions

Both studies on 20-HETE and EETs demonstrate the use of metabolomic profiling as a promising tool to gain a more comprehensive understanding of the biological processes. An increased sEH activity has been demonstrated in an animal model of MI, supporting the notion that sEH may play an important role in the progression of post-ischemia remodeling. However, increased expression level of this enzyme has not been directly detected in the heart. Further studies to explore the mechanism by which sEH activity is dysregulated in MI and possible involvement of other organs such as liver and kidney may help to shed new light on the molecular defects in the pathogenesis of myocardial failure. Moreover, In order to definitively determine the best therapeutic utility for sEHIs, future studies to evaluate the

potential interactions of sEHIs with other pharmaceuticals are warranted. It has been shown that regulation of sEH is intimately tied to the rennin-angiotensin-aldosterone system (RAAS) in animal models of hypertension and cardiac hypertrophy. sEHIs also synergize with COX-2 inhibitors and other modulators of the arachidonic acid cascade to exert anti-inflammatory effects. Thus, the combination of sEHIs and angiotensin converting enzyme inhibitors or COX inhibitors may provide powerful combination drug therapies with more favorable side effect profiles. Since heart failure is a complex clinical syndrome with diverse etiology and a wide array of pathophysiology, in order to translate the observed beneficial effects of sEHIs into clinical intervention in patient care, additional information is needed to identify whether the observed beneficial effects can be generalized to other causes of heart failure, such as idiopathic dilated cardiomyopathy, drug-induced heart failure,

Heart failure and its co-morbidities represent a major market and one of the paths to the clinic for sEHI that is best supported by mechanism, animal studies and human data. However, the length, high cost, and high risk of clinical trials for heart failure make this path unattractive to many pharmaceutical companies. Investigational new drug (IND) status for an sEHI could permit investigator initiated clinical trials to address this problem. Finally, other oxylipins apart from the ω-6 arachidonic acid metabolites may be relevant in cardiovascular disease. For example, the ω-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) accumulate in the heart [50] and the epoxides of EPA and DHA are analogs of the EETs. In fact, DHA and EPA epoxides share some of the vasoactive and anti-inflammatory properties of the EETs in vitro and in some cases have been shown to be more potent [51, 52]. DHA and EPA epoxides are, in general, better substrates for sEH than the EETs [Morisseau and Inceoglu, unpublished], so it is possible that some of the cardioprotective effects of sEH inhibition are due to reduction in DHA and EPA epoxide metabolism in the heart. Intervention studies with ω-3 lipids could test the hypothesis that these natural products have a protective effect on cardiac hypertrophy.

In summary, metabolomic profiling has been shown to not only identify a potential marker of risk or effect of rofecoxib and possibly other drugs, but also to demonstrate paths to mitigate the risk of these valuable pharmaceuticals. In addition, metabolomic profiling expanded our knowledge of the role of eicosanoids in the progression of cardiac hypertrophy. This knowledge from profiling pointed to inhibitors of the sEH as possible therapeutic agents for the prevention or even treatment of this and other cardiovascular diseases. Over the past few years, the use of sEHIs in animal models have demonstrated that sEHI have therapeutic potential in a broad range of cardiac diseases, many of which are co-morbidities with hypertrophy. Although possible side effects associated with the inhibition or genetic deletion of sEH have been reported [53, 54], the data obtained from several laboratories employing animal models of cardiac hypertrophy and ischemia/reperfusion support the notion that sEHIs and possibly EET mimics represent promising therapeutic targets for combating detrimental cardiac remodeling, heart failure and related diseases.

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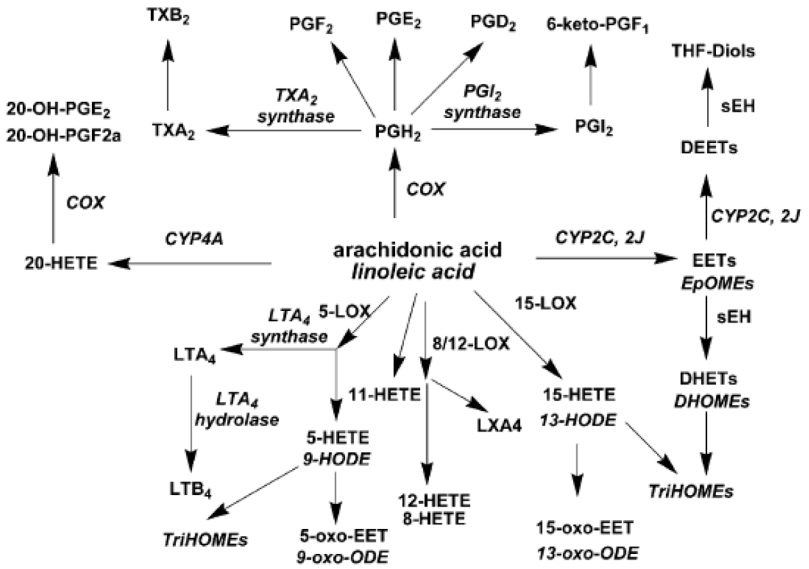

Figure 1. Diagram illustrating the metabolic pathways for arachidonic acid and linoleic acid

Arachidonic acid is metabolized through three enzymatic pathways. The cyclooxygenase (COX) pathway produces prostanoids. The lipoxygenase (LOX) pathway yields monohydroxy compounds and leukotrienes, while the cytochrome P450 (CYP) epoxygenase pathway generates hydroxy and epoxyeicosanoids.