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Drug-Disease Interaction: Effect of Inflammation and Nonsteroidal Anti-inflammatory Drugs on Cytochrome P450 Metabolites of Arachidonic Acid

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ABSTRACT

Inflammatory conditions increase cardiovascular (CV) risk. Some nonsteroidal anti-inflammatory drugs (NSAIDs) that are used to treat pain and inflammation are also associated with CV complications. Inflammation, but not NSAIDs, disrupts the balance of vasodilator and vasoconstrictor components of the renin-angiotensin system within the heart. Herein, we report the effect of both inflammation and NSAIDs (rofecoxib, celecoxib, and meloxicam) on the physiologically active cytochrome P450 metabolites of arachidonic acid (ArA) in the rat with adjuvant arthritis. After oral administration of 7 daily therapeutically equivalent doses of NSAIDs or vehicle, the anti-inflammatory response, as well as the ArA metabolites and drug concentrations in plasma, heart and kidneys were assessed. Inflammation in the form of adjuvant arthritis caused a significant tissue-dependent imbalance of ArA metabolites by elevating the ratio of cardiotoxic 20-hydroxyeicosatetraenoic acid over cardioprotective epoxyeicosatrienoic acids in the heart, and reducing the ratio in the kidney. The observed imbalance was augmented by cardiotoxic rofecoxib but not by other examined NSAIDs with known milder cardiotoxicity. The cardio-renal toxicity of NSAIDs with known severe CV side effects may be due to altered cytochrome P450–mediated ArA acid metabolism. The ArA metabolism profile may be a marker of NSAIDs safety and toxicity.

Introduction

Inflammation is associated with many conditions including arthritis, inflammatory bowel disease, cancer, mental disorders,[1,2] and increased cardiovascular (CV) risks.[3] Some of the nonsteroidal anti-inflammatory drugs (NSAIDs) that are used to treat inflammation are also reported to contribute to the elevation of the CV risks with severe consequences.[4] The mechanisms behind these CV complications are mainly unknown. However, as it is now known that NSAIDs are heterogeneous, their potency to elevate CV risks range from marginal (e.g., meloxicam) to severe (e.g., diclofenac and rofecoxib).[5] Recently, we have reported that in an experimental model of arthritis, inflammation impairs the balance of vasodilator/vasoconstrictor components of renin-angiotensin system (RAS) within the heart.[6] The RAS is a major regulator of human physiology and has a key role in the CV homeostasis.[7] Interestingly, NSAIDs appear to be void of significant effects on RAS, instead, rather be able to restore the imbalances that are caused by inflammation.[6] This suggests that mechanisms other than imbalances in the RAS components are involved in the cardiotoxic effects of NSAIDs.

NSAIDs exert their effects mainly by blocking prostaglandin synthesis from its precursor arachidonic acid (ArA). ArA, a phospholipid component of the cell wall, is cleaved by phospholipase enzyme and metabolized by cyclooxygenase (COX), lipoxygenase, and cytochrome P450 (CYP) to produce lipid metabolites such as prostanoids (prostaglandins, prostacyclin, and thromboxane), leukotriene, and eicosanoids, respectively.[8,9] The COX and lipoxygenase pathways of ArA metabolism are well characterized as they are the targets for anti-inflammatory drugs. However, the CYP metabolism pathway of ArA has received attention only since the discovery that its products are biologically active and are involved in many physiological and pathophysiological processes.[8] Enzymes CYP4A and CYP4F metabolize ArA by ω-hydroxylation and

Abbreviations used: AA, adjuvant arthritis; ArA, arachidonic acid; COX, cyclooxygenase; CV, cardiovascular; CYP, cytochrome P450; DHT, dihydroxyeicosatrienoic acid; EET, epoxyeicosatrienoic acids; T-EET, total EET; HETE, hydroxyeicosatetraenoic acid; NSAID, nonsteroidal anti-inflammatory drug; RAS, renin-angiotensin system.

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epoxidation to produce hydroxyeicosatetraenoic acids (HETEs) and epoxyeicosatrienoic acids (EETs).[10] 20-HETE, a potent vasoconstrictor, plays important roles in the regulation of the vascular tone in various tissues. Different pathological conditions such as ischemic cerebrovascular diseases, hypertension, diabetes, kidney diseases, and cancer are linked to changes in 20-HETE production.[11,12] EETs, formed by CYP2C and CYP2J subfamily, are another important group of ArA metabolites with potent vasodilatory and anti-inflammatory effects.[13] They are shown to induce smooth muscle hyperpolarization and relaxation. Soluble epoxide hydrolase metabolizes EETs to dihydroxyeicosatrienoic acids (DHTs) with less vasodilatory activity than the parent EETs.[14] Studies suggest localized site-specific expressions, as well as the paracrine and autocrine nature for CYP metabolites of ArA.[15-17]

Acute inflammation induced by injection of lipopolysaccharide has shown to influence 20-HETE/EET balance in plasma. However, its effect on the heart concentration of the ArA metabolites is controversial. An increased cardiac 20-HETE:EET was initially reported[18] that others,[19] including us (unpublished data), were unable to reproduce. In addition, with regard to NSAIDs, Liu et al.[20] reported a pronounced increase in the plasma concentration of 20-HETE in rofecoxib-treated healthy mice. Rofecoxib, a highly selective inhibitor of COX-2, was withdrawn due to its contribution in increased CV risks.[4] The potential additive effect of NSAIDs on the ArA metabolic profile in systemic inflammation is unknown.

The present study was carried out with 2 novel aims: to investigate the effect of (1) systemic inflammation and (2) a selected group of NSAIDs on ArA metabolite levels in plasma, heart, and kidney of rats with adjuvant arthritis (AA). We chose 3 NSAIDs with the following reported extent of cardiotoxicity: rofecoxib > celecoxib = meloxicam[21] to test our hypothesis that NSAIDs with high potential to increase CV risks, for example, rofecoxib, cause imbalances in ArA metabolism.

Material and Methods

Materials

The ArA metabolites were purchased from Cayman Chemical Company (Ann Arbor, MI). They consisted of analogs of HETEs, EETs, and DHTs. The fluorescent label, 2-(2,3-naphthalimino)ethyl-trifluoromethanesulfonate (NE-OTf) was obtained from Molecular Probes (Eugene, OR). Celecoxib was a gift from Pfizer (Harbor Beach, MI), and rofecoxib was obtained from Yick-Vic Chemicals & Pharmaceuticals Ltd. (Kowloon, Hong Kong), meloxicam from Unichem Laboratories Ltd (Bombay, India). Lowry protein assay kit obtained from Bio-Rad Laboratories (Hercules, CA).

Adjuvant Arthritis Rat Model

The study protocol was approved by the Health Sciences Animal Care and Use Committee of University of Alberta, Edmonton, Canada (Permit #276). Adult male Sprague-Dawley rats weighing 230-250 g, purchased from Health Sciences Laboratory Animal Services, and were housed under ambient temperature and ventilation with 12 h day and night cycles. Rats were kept in standard cages with free access to drinking water and regular rat chow. After 48 h of acclimatization, rats were randomly divided into healthy-control (n = 7) and AA groups. AA rats were further divided to AA-control, AA-rofecoxib, AA-meloxicam, and AA-celecoxib (n-7/group). This study was carried out twice, first with 3/group, and then with 4/group. Rats were anaesthetized with isoflurane/oxygen and injected at the tail base with of single doses of either 50 mg/mL *Mycobacterium butyricum* in 0.2 mL squalene solution (Difco Laboratories, Detroit, MI) (AA rats) or pyrogen-free sterile saline (healthy-control).

Subsequently, rats were monitored daily and assessed for emergence of AA by assigning an arthritis index score to each. Arthritis index is a macroscopic scoring system[22]: for each hind paw on a 0-4 scale, 0 = no sign; 1 = single joint involved; 2 = more than 1 joint and ankle involved; 3 = several joints and ankle involved with moderate swelling; 4 = involvement of several joints and ankle with severe swelling. For each forepaw on a 0-3 scale, 0 = no sign; 1 = single joint involved; 2 = more than 1 joint and wrist involved; 3 = involvement of wrist and joints with moderate-to-severe swelling. The index was calculated by adding all of the above scores together to attain a maximum of 14. An arthritis index score of $\geq$5 was considered infliction of the disease which was evident typically in 9-12 days after adjuvant injection. Subsequently, rats were either treated with NSAIDs or euthanized to minimize their suffering. Specimens collected from 5 of these animals were also used in another study.[6]

Serum nitrate and nitrite were quantified by using Griess reagent according to a published method.[23]

Dosage Regimens, Treatment, and Sampling

The NSAID doses were those that produced area under plasma concentration-time curves (AUCs) equivalent to those reported following administration of maximum therapeutic doses recommended for humans.[24] To calculate the latter, we obtained AUCs in both humans and rats from either our own data files or those reported in the literature. The doses in mg/kg/d were 10, 0.5, and 15 for rofecoxib, meloxicam, and celecoxib, respectively, suspended in PEG200 and administered orally via oral gavage once daily for 7 days. The healthy-control and AA-control groups were dosed with drug-free PEG200. On day 8 after the treatment, rats were anesthetized with isoflurane/oxygen (0.75/2%) and blood samples were collected by cardiac puncture. Subsequently, heart and kidneys were rapidly removed, washed with saline to remove blood, and stored. Serum was separated from a portion of the collected blood for nitrite analysis. After centrifugation for 10 min at 2500 $\times$ g, the plasma was separated and stored. All samples were stored at -80°C until analysis.

Assays

ArA Metabolites

The CYP metabolites of ArA were measured by validating a previously published reversed phase HPLC assay.[25] Briefly, specimens were thawed to 4°C, and aliquots of approximately 300 mg of the heart or kidney tissue were weighted, homogenized, and centrifuged in glass tubes containing methanol and formic acid. To the homogenized tissue samples or 0.6 mL of plasma were added 16-hydroxydecanoic acid as internal standard. The resultants was extracted using reversed-phase cartridges, centrifuged, dried, reconstituted in anhydrous acetonitrile and derivatized with 2-(2, 3-naphthalimino) ethyl-trifluoromethane sulphonate before injecting onto HPLC.

The method[25] was validated to quantify 13 ArA metabolites. However, we analyzed only the major metabolites that included 20-HETE, 8,9-EET, 11,12-EET, 14,15-EET, 5,6-DHT, 8,9-DHT, 11,12-DHT, and 14,15-DHT. Total-EET (T-EET) was calculated by adding 8,9-EET, 11,12-EET, and 14-15 EET.

NSAIDs

For the NSAIDs concentration analysis, plasma was separated in Eppendorf tubes, snap frozen in liquid nitrogen, and stored at -80°C until analyzed. NSAIDs were measured in 0.1 mL plasma