

## Mechanisms of Cyclooxygenase-2 Inhibition and Cardiovascular Side Effects—The Plot Thickens

Perspective on Duffield-Lillico et al., p. 322

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Cyclooxygenase (COX)-2 is a major player in inflammation and cancer. It is one of two COXs that convert arachidonic acid to a common intermediate in the production of prostaglandins and thromboxane. COX-2 is inhibited by nonselective COX inhibitors such as aspirin, ibuprofen, and sulindac as well as by the COX-2-selective inhibitors celecoxib and rofecoxib. Inflammation is an important contributor to cancer development, and COX inhibitors, whether selective for COX-2 or not, exhibit chemopreventive activity in animal models and patients. Celecoxib, rofecoxib, and sulindac showed impressive reductions in polyp recurrence but also caused cardiovascular side effects in colorectal clinical prevention trials (1–4). Subsequent studies revealed that the adverse cardiovascular effects are mechanism based (i.e., they result from the inhibition of COX-2) and that they are especially relevant to individuals with preexisting risk factors for coronary artery disease (1, 5). This mechanistic basis is the focus of this perspective, whereas two other articles in this issue of the journal address the clinical basis of the adverse cardiovascular consequences of COX-2 inhibitors (6, 7), which led to the withdrawal of rofecoxib from the market and a significant reduction in the use of celecoxib.

Cigarette smoking has been reported to cause an increase in the production of prostaglandins in the lung, which is manifested by the excretion of the major urinary metabolite of prostaglandin E<sub>2</sub>, called PGE-M (8). In this issue of the journal, Duffield-Lillico et al. (9) report that the increase in urinary PGE-M is due to the induction of COX-2. Using a rigorous, mass spectrometry-based analytic method, they examined the effect of celecoxib treatment on the levels of PGE-M in never smokers, ex-smokers, and current smokers. As expected, the levels of PGE-M were higher in the current smokers and ex-smokers. Celecoxib significantly decreased urinary PGE-M in all groups, but the effect was much greater in smokers and ex-smokers, who exhibited high baseline levels of the metabolite. These findings suggest that the elevated levels of PGE-M excreted by smokers result from the induction of COX-2, probably in the lung. Increased levels of COX-2 have been observed in patient lung cancer speci-

mens and in airway cells from patients with chronic obstructive pulmonary disease (10, 11). The effect of celecoxib on PGE-M levels allows the conclusion that smoking-induced COX-2 levels stimulate the production of lipid mediators of pulmonary inflammation.

This is a nice demonstration of the use of validated biomarkers to probe mechanism of drug action in patients. The result, although solid and informative, is not too surprising given the sensitivity of the COX-2 gene to induction by a broad range of stimuli. However, Duffield-Lillico et al. also monitored the urinary levels of a major metabolite of the 5-lipoxygenase (5-LOX) pathway and got a real bonus. 5-LOX is another enzyme of arachidonic acid oxygenation that catalyzes the first step in a cascade leading to the production of leukotrienes. Leukotrienes are established mediators of inflammation and allergy. Leukotriene B<sub>4</sub> is a powerful chemotactic agent for neutrophils, and leukotrienes C<sub>4</sub> and D<sub>4</sub> are powerful bronchoconstrictors and vasoconstrictors. Used for the treatment of asthma, the drug montelukast is an antagonist of the leukotriene C<sub>4</sub>/D<sub>4</sub> receptor. Leukotrienes C<sub>4</sub> and D<sub>4</sub> are metabolized to leukotriene E<sub>4</sub> (LTE<sub>4</sub>), which is excreted in urine. LTE<sub>4</sub> is a ready biomarker of the action of 5-LOX. There is a growing body of evidence linking leukotrienes to cardiovascular disease (12). For example, polymorphisms in genes associated with leukotriene biosynthesis are associated with an increased risk of myocardial infarction, stroke, and atherosclerosis (13–16); elevated urinary levels of LTE<sub>4</sub> have been reported for patients with acute myocardial infarction, unstable angina, or coronary artery disease before and shortly after bypass surgery (17, 18).

Duffield-Lillico et al. measured the levels of LTE<sub>4</sub> and PGE-M in the same series of patients. As with the PGE-M measurements, baseline levels of LTE<sub>4</sub> were positively associated with smoking status (i.e., they were higher in current smokers). This is another indication that smoking induces pulmonary inflammation. However, in contrast to the decrease in PGE-M in smokers treated with celecoxib, the levels of LTE<sub>4</sub> increased in smokers treated with the drug. One interpretation of this observation is that when arachidonic acid is not used by the COX pathway, it is shunted into the 5-LOX pathway (Fig. 1). This shunt has been proposed for many years, but this is the first evidence that it occurs in human beings. The increase in levels of LTE<sub>4</sub> occurred only in individuals with high PGE-M, which suggests that the 5-LOX exists in a location close to the tobacco smoke-induced COX-2.

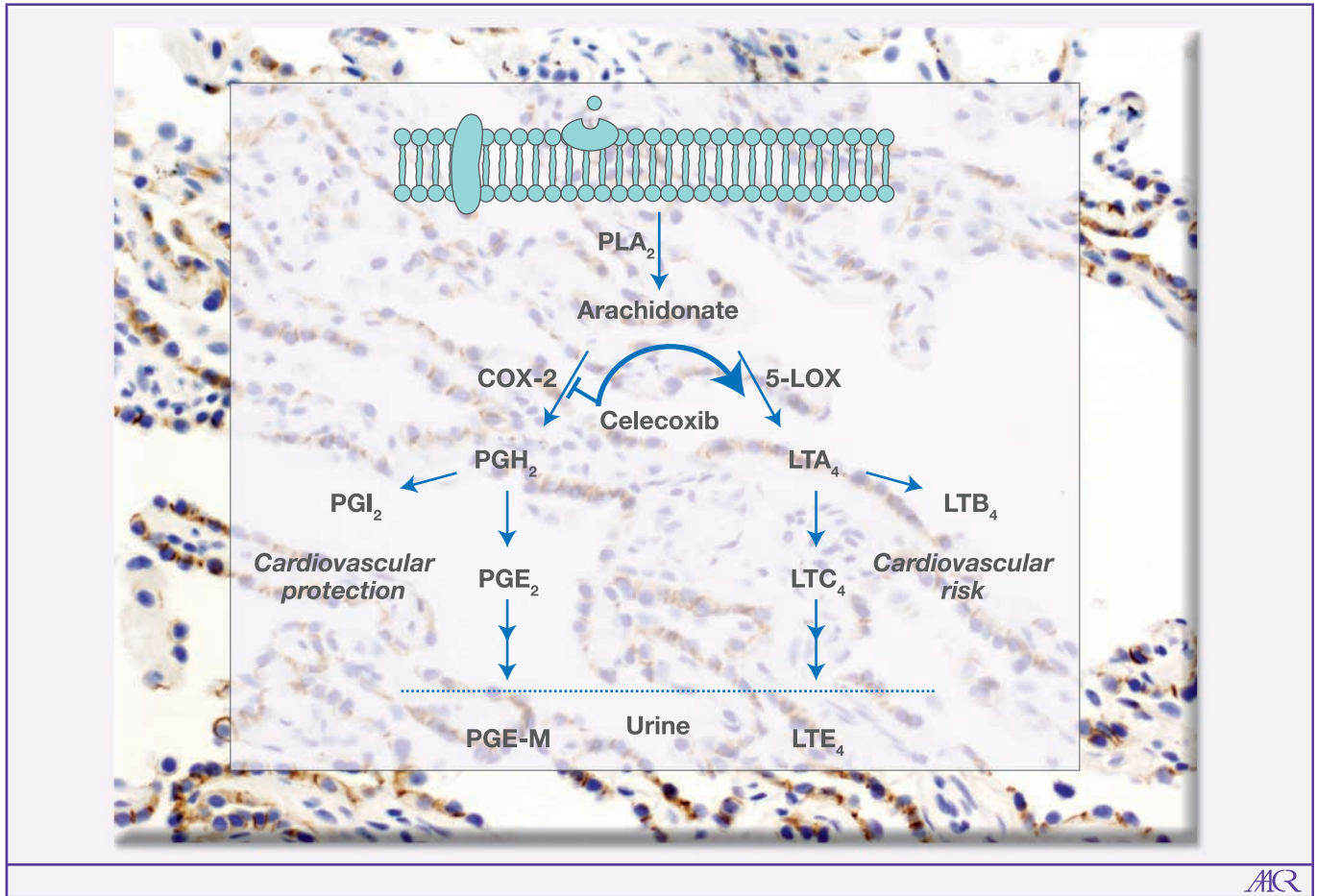
In addition to providing evidence for the redirection of arachidonate from COX-2 to 5-LOX (i.e., from prostaglandin biosynthesis to leukotriene biosynthesis), these observations

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**Fig. 1.** Celecoxib shunts arachidonic acid from the COX-2 pathway to the 5-LOX pathway. Arachidonate, which is released from cell membrane stores (top; the membrane shows protein structures unrelated to this shunt) by the action of phospholipase A<sub>2</sub> (PLA<sub>2</sub>), is oxygenated by COX-2 or 5-LOX. Some of the products of the COX-2 pathway (most notably, PGI<sub>2</sub>) exert cardiovascular protective activities, whereas the products of the 5-LOX pathway are associated with enhanced cardiovascular risk. Blocking COX-2 action with an inhibitor such as celecoxib increases the flux of arachidonic acid through the 5-LOX pathway. The oxygenation of arachidonate via the two pathways can be monitored by the levels of the urinary biomarkers PGE-M and LTE<sub>4</sub>. The figure does not mean to imply that COX-2 and 5-LOX are located in the same cells.

may provide fresh insights into the cardiovascular toxicity of COX-2 inhibitors. Multiple factors have been associated with COX-2 inhibitor-associated cardiovascular toxicity, but all involve a decrease in the production of the antithrombotic and antiatherogenic prostaglandin, prostaglandin I<sub>2</sub> (PGI<sub>2</sub>), resulting from the inhibition of COX-2 in the vessel (e.g., coronary artery) wall. This process was shown in 1999 by McAdam et al. using a rigorous mass spectrometric method. They showed that celecoxib (and later, rofecoxib) selectively reduced the secretion of the major urinary metabolite of PGI<sub>2</sub> in people taking the drug (19). This was a stunning result at the time because the link between COX-2 and vascular PGI<sub>2</sub> biosynthesis had not been fully appreciated. The prediction by McAdam et al. that COX-2 inhibition might be associated with cardiovascular events was borne out in the placebo-controlled polyp prevention trials mentioned earlier. The discovery by Duffield-Lillico et al. that celecoxib increases the urinary excretion of leukotrienes associated with cardiovascular risk suggests that this effect may contribute to increased cardiovascular events in individuals taking COX-2-selective and some other nonsteroidal anti-inflammatory drugs.

These findings need to be confirmed, and one can imagine the design of many parallel studies with different inhibitors as well as experiments in mouse models to test the importance of 5-LOX action in cardiovascular toxicity induced by COX-2-selective inhibitors. Nevertheless, the findings provide potentially important new insights into the pharmacology of COX inhibitors and the complexity of selectively modulating signal transduction by lipid mediators. Furthermore, the study underscores the value of high-quality biomarkers in clinical investigations. Last, the findings may have therapeutic implications. If the cardiovascular risk of COX-2 inhibition is due to the reduction of PGI<sub>2</sub> biosynthesis and an increase of leukotriene biosynthesis, simultaneous modulation of the COX-2 and 5-LOX pathways may reduce the cardiovascular risk of COX inhibitors and thus enable their full chemopreventive potential to be realized. This combination therapy may even lead to increased chemopreventive activity, as seen in animal models (20).

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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