LETTER

COX-2, the dominant source of prostacyclin

In a recent issue of PNAS, Kirkby et al. (1) claimed that cyclooxygenase (COX)-1, not COX-2, is the dominant source of prostacyclin (PGI_2). This conclusion was based on experiments using a flawed approach to estimating enzyme activity and PGI_2 formation and a selective omission of data in the literature.

Kirkby et al. (1) used an immunoassay of 6-keto prostaglandin (PG)$F_{1\alpha}$ to reflect PGI_2. However, their reported levels (~150 ng/mL) were orders of magnitude higher than when 6-keto $PGF_{1\alpha}$ is measured (<3 pg/mL) in plasma by mass spectrometry (MS) (2). The source of immunoreactivity in the study by Kirkby et al. was unknown. Polyclonal antibodies against COX-1 and COX-2 were used to compare relative expression in murine vascular tissue. Although Kirkby et al. detected COX-1, they did not see a COX-2 signal and concluded that this isoform was not expressed. However, they failed to demonstrate that the antibody batches, dilutions, and exposure settings used in these experiments have similar sensitivities for detecting COX-1 vs. COX-2. Other limitations of their study included quantitative comparisons of immunohistochemistry to infer differential enzyme activity and varied use by COXs of high concentrations of substrate.

We first reported that COX-2 was the dominant contributor to PGI_2 biosynthesis, measuring its urinary metabolite, 2,3-dinor 6-keto $PGF_{1\alpha}$ (PGIM), by MS in volunteers administered nonsteroidal anti-inflammatory drugs (NSAIDs) that differed in selectivity for inhibition of COX-2. Subsequently, we confirmed that PGIM was markedly suppressed by global deletion or inhibition of COX-2 in mice, consistent with results of Kirkby et al. (1). However, the authors claimed PGIM is not reflective of vascular PGI_2, reporting that infusion of a vascular stimulant, bradykinin, evokes COX-1–derived immunoreactive 6-keto $PGF_{1\alpha}$, but not PGIM, in mice. However, they failed to cite our previous work that systemic infusion of either PGI_2 (3) or bradykinin (4) markedly increases urinary PGIM (and 6-keto $PGF_{1\alpha}$ from which it is derived) in humans.

Kirkby et al. (1) failed to mention that deletion of COX-2 in vascular, smooth muscle cells, or both depressed urinary PGIM, providing direct genetic evidence for the role of vascular COX-2 in PGI_2 production (5). They also ignored the functional consequences of vascular deletion of COX-2, a predisposition to thrombosis and hypertension, or that urinary PGIM (alone among the major metabolites of PGs was suppressed in these mutants) inversely correlated with the rise in blood pressure (5), consistent with the hypothesis that the hypertensive phenotype is a consequence of disruption of COX-2–dependent formation of PGI_2.

Disruption of rodent COX-2–derived PGI_2 in vascular cells and cardiomyocytes has recapitulated all elements of the cardiovascular hazard attributable to NSAIDs in humans, and data consistent with our original hypothesis have been obtained from human genetics, other animal models, observational studies, randomized comparisons among NSAIDs, and eight placebo-controlled trials of NSAIDs designed to inhibit selectively COX-2. This represents the most diversified body of data supportive of a mechanism of drug action ever assembled and stands in contrast to the contention by Kirkby et al. (1) that a molecular basis for the cardiovascular hazard from NSAIDs is unknown; COX-2, not COX-1, is the dominant source of PGI_2 in vivo.

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Author contributions: E.R., Y.Y., T.G., and G.A.F. wrote the paper.

The authors declare no conflict of interest.

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