HHS Public Access

Author manuscript

Circ Res. Author manuscript; available in PMC 2019 February 16.

Published in final edited form as:

Circ Res. 2018 February 16; 122(4): 537–539. doi:10.1161/CIRCRESAHA.118.312616.

Urinary prostaglandin metabolites: An incomplete reckoning and a flush to judgement

Tilo Grosser[1], **Ali Naji**[2], and **Garret A FitzGerald**[1]

[1]Institute for Translational Medicine and Therapeutics

[2]Department of Surgery, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104

Keywords

prostacyclin; thromboxane; biomarkers

Prostaglandins are chemically unstable bioactive lipid autacoids, acting locally to exert diverse biological functions. An indirect approach to estimating their biosynthesis has been to measure biologically inert, but chemically stable metabolites using mass spectrometry.[1] Prostaglandins and thromboxane (Tx) A_2 are formed by the cyclooxygenase (COX) enzymes from arachidonic acid, an unsaturated fatty acid constituent of cell membranes. Most cell types make one of two dominant products of these enzymes. Although many prostaglandins and related lipids, such as leukotrienes, may perturb cardiovascular function, there has been a particular interest in prostacyclin (PGI_2), the major COX product of vascular cells and TxA_2formed by platelets. While the simplistic concept of a “balance” between these products is naïve,[2] they are amongst the many endogenous modulators of platelet and vascular function. Most insights into the in vivo biology and pharmacology of the prostaglandin pathway have derived from the measurement of metabolites, particularly in urine.[3] Given how indirect is this approach, it is remarkable how informative it has turned out to be. However, there are a few fundamental concepts intrinsic to interpretation of such data.

Firstly, there is a striking discordance between the capacity of cells to make these lipids and their actual formation in vivo, even under conditions in which biosynthesis is augmented and of functional relevance. For example, the capacity of platelets to make TxA_2, reflected by measurement of its more stable hydrolysis product TxB_2 in serum, is in the order of 300 - 400ng/ml. By contrast, maximal estimates of plasma concentrations in vivo are ~2pg/ml.[4] Capacity related measurements have been invaluable biomarkers, both in vitro and ex vivo, of inhibitory drug effect as exemplified in the dose dependent suppression of serum TxB_2 by aspirin.[5] In these circumstances, they correlate quite nicely with measurements of urinary Tx metabolites (M) in vivo.[5,6] However, the discordance between biosynthetic rates and tissue

Corresponding Author: Garret FitzGerald, Institute for Translational Medicine and Therapeutics, Translational Research Center 10-116, University of Pennsylvania, Philadelphia, PA 19104, Phone: (215) 898 1185, garret@upenn.edu.

Conflict of Interest Statement: T.G., A.N. and G.A.F declare no conflicts of interest.

capacity to form Tx means that elevated urinary TxM, but notserum TxB_2, reflects augmented Tx biosynthesis during episodes of cardiac ischemia[7] or concurrent with therapeutic thrombolysis,[8] both settings where the clinical efficacy of aspirin implies functional relevance. This discordance between capacity and endogenous rates of biosynthesis also explains how artifacts of venipuncture and ex vivo platelet activation confound the use of TxB_2 or the hydrolysis product of PGI_2, 6-keto $PGF_{1\alpha}$, in plasma as indices of biosynthesis.[1,9]

These caveats render urine an attractive target for biomarker evaluation, given that it is acquired noninvasively. However, it also comes with long recognized[1] limitations. We know from infusion studies that altered levels of PGI_2 and TXB_2 in the circulation are rapidly and sensitively reflected by changes in their urinary metabolites. Similarly, they rapidly and reversibly reflect both therapeutic and experimental mechanical perturbation of the vasculature. However, as with biomarkers in plasma, it is usually impossible to attribute definitively a tissue of origin to metabolites in urine.[1] An exception is in the case of the anucleate platelet. Thus, in healthy volunteers, low dose aspirin suppresses the two major TxM (11-dehydro TxB_2 and 2,3 dinor TxB_2) by ~80% and, recovery corresponds to platelet turnover time.[10] Thus, platelets are the major source of TxM in healthy people. Increased platelet activation in vivo would be expected to result in an increase in urinary TxM, as indeed it does.[7,8] However, given the discordance with tissue capacity, augmented formation of Tx by another tissue source, for example activated macrophages, would be expected to increase urinary TxM despite suppression of platelet Tx by low dose aspirin and that is what we see in smokers.[11] Thus, *augmented* formation by another tissue source, such as the lung or kidney, would be expected to supersede the contribution to TxM from platelets that dominates under physiological conditions and is increased in syndromes of platelet activation. More definitive information about tissue of origin can be gleaned by tissue selective gene deletion in mice. Thus, whereas suppression of urinary PGIM in volunteers by celecoxib and rofecoxib revealed an unexpected contribution of COX-2 to their biosynthesis and the prediction of a cardiovascular hazard[2], depletion of COX-2 in vascular cells in mice depressed the same metabolite and resulted in accelerated thrombogenesis and hypertension.[12]

In the case of the kidney, we have known for more than 25 years of its capacity (along with liver and lung) to make PGIM and TxM.[13] Under physiological circumstances, most urinary TxB_2 derives from the kidney, most TxM from the systemic circulation. However, if an additional renal source of Tx production is activated during physiological challenges or disease, it would be expected to become the dominant source of both urinary TxB_2 and TxM.[2,13]

Given these precautions to interpretation, the use of urinary metabolites of TxA_2 and PGI_2 have contributed to the development of low dose aspirin for cardioprotection, the rationalization and assessment of combining aspirin with thrombolytic drugs and the prediction of the cardiovascular hazard from other NSAIDs, such as rofecoxib.

In this issue of Circulation Research, Mitchell et al challenge this body of evidence based on their studies of a single patient with a genetic deficiency in prostanoid formation.[14] Despite

the stridency of their claims, such a fundamental reconsideration of the literature would seem to be premature.

The patient and a sibling had been previously diagnosed with a homozygous loss-of-function mutation in the gene PLA2G4A, which encodes a member of the cytosolic phospholipase (PL) A_2 group IV family.PLA_2 hydrolyzes membrane phospholipids to release arachidonic acid, the ubiquitous substrate for COX-dependent prostanoid formation. Both individuals presented with cryptogenic multifocal ulcerating stenosing enteritis attributed to the genetic defect. Markedly decreased urinary TxM and PGIM levels were detected consistent with the expected deficiency of formation of all prostaglandins and specifically under “basal” circumstances, with a depression of TxA_2 and PGI_2 formation in platelets and endothelial cells. Spanning a 40 year history, both patients experienced severe complications from extensive ulcerative disease of the entire gastrointestinal tract, resulting in chronic renal failure in one sibling. This patient eventually underwent kidney transplantation from an unrelated donor – with intact PLA_2 function – affording a unique opportunity to measure the contribution of the transplanted kidney to urinary PGIM and TxM. The authors report that urinary PGIM and TxM were very low prior to transplantation and rose to levels comparable to those in a healthy control group, 1-3 months following successful transplantation, providing human genetic evidence that the kidney can be a dominant tissue source of these urinary biomarkers. Although this is an unusual opportunity to study tissue dependent eicosanoid formation in a human, two factors confound the conclusions drawn by the authors from this study.

Firstly, no data exist on the elimination kinetics of PGIM and TxM in renal failure, the authors observed a 25% reduction in PGIM plasma concentrations following transplantation consistent with an increase in PGIM clearance when kidney function was restored. Furthermore, in renal failure urine creatinine concentrations reflects largely tubular secretion rather than glomerular filtration and this may have confounded the normalization of PGIM and TxM to urine creatinine. Also, differences in muscle mass between healthy volunteers and a patient with severe disease burden may have confounded normalization to urine creatinine. A more appropriate approach would have been the comparison of the patient's pre-transplantation TxM and PGIM measurements with an age, sex and weight matched group of patients with chronic renal failure but intact PLA_2 function. The post-transplantation measurements in the PLA_2 deficient patient would have best been compared with data obtained in matched kidney transplantation patients with intact PLA_2.

More important is the fundamental issue that an increase in urinary TxM and PGIM by transplantation of a tissue with the capacity to form these metabolites into a patient with PLA_2 deficiency is entirely unsurprising given the discussion above[1,13] and is irrelevant to the appropriate use of this approach to monitoring platelet and vascular function. The long recognized metabolic capacity of the kidney to form these metabolites results, as expected, in it being the major contributor to their formation when transplanted into a patient whose genetic deficiency disables prostaglandin formation. However, this does not challenge the contribution of platelet vessel wall interactions as the dominant source of these metabolites under physiological conditions in healthy people or the utility of these metabolites as biomarkers of platelet dependent vascular occlusion.

Aside from the intrinsic ability of kidney to form them, prostanoid formation may have been further increased in this patient by some degree of renal inflammation. While clinical acute kidney allograft rejection on potent immunosuppressive agents such as tacrolimus is infrequent, minor degrees of inflammation not qualifying as “rejection” are a common finding in biopsies. Indeed, subclinical inflammation with stable kidney function despite immune suppression is thought to affect adversely long-term graft survival.[15] In a previous report, the authors observed an exaggerated inflammatory response in the PLA_2 deficient patient studied here. Whole blood and cultured endothelial cells exhibited markedly greater cytokine responses to LPS and other inflammatory agents as compared with matched controls. However, neither inflammatory markers nor the results of an allograft biopsy have been reported in the transplant patient. Thus, it seems plausible that a substantial fraction of the TxM and PGIM derived from the kidney allograft is produced by an inflammatory process rather than solely by normal renal function.

In summary, the judicious interpretation of urinary prostaglandin metabolite data has yielded predictive and therapeutic insights over the past 40 years. Here, Mitchell et al. provide human genetic evidence that kidney allograft can produce substantial amounts of TxM and PGIM. The renal capacity to form these metabolites has long been known and this can readily be augmented by inflammation. It is therefore unsurprising that metabolite formation would largely reflect renal biosynthesis when a kidney is transplanted into a patient with a markedly depressed capacity to form prostaglandins in other tissues. These observations do not question the utility of urinary TxM and PGIM as indices of platelet-vascular interactions but rather remind us of how important in this field has been rigorous experimental design and careful interpretation of data.

Acknowledgments

Sources of Funding: Supported by NIH/NHLBI grant 1U54 HL117798

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