Analysis of Prostacyclin and Thromboxane Biosynthesis in Cardiovascular Disease

Garret A. FitzGerald, M.D., Anders K. Pedersen, M.D., and Carlo Patrono, M.D.

The appreciation that two oxygenated metabolites of arachidonic acid, prostacyclin (PGI₁) and thromboxane A₂ (TXA₂), have potent and contrasting effects on vascular tone and platelet function in vitro has prompted attempts to define their importance in human vascular disease. Both compounds are formed from arachidonic acid by the enzyme cyclooxygenase via cyclic endoperoxide intermediates, PGG₃ and PGH₂, TXA₂, the major cyclooxygenase product in the platelet, is a vasoconstrictor and potent stimulus to platelet aggregation in vitro. PGI₁, a vasodilator and inhibitor of platelet aggregation, is the predominant product of the same enzyme in vascular endothelial cells. The development of techniques to study platelet function in vivo and ex vivo preceded the discovery of these compounds, and the application of these tests suggests that increased platelet activation may well accompany vascular occlusive events in man. Thus, it seems reasonable that PGI₁ and TXA₂ might play an important role in this and other syndromes of disordered platelet-vascular homeostasis in man. Support for this concept relies upon the development of methods to measure PGI₁ and TXA₂ in human biological fluids. However, both PGI₁ and TXA₂ have extremely short half-lives and are rapidly cleared from the bloodstream. Furthermore, arachidonic acid release from biological membranes (and thus synthesis of PGI₁ and TXA₂) is likely to be phasic rather than continuous. This phenomenon also renders measurement of these products highly liable to artifact when performed in media obtained by invasive sampling techniques, such as plasma. Finally, there are the technical difficulties involved in devising assays of requisite sensitivity and specificity.

Three approaches have been used: bioassay, radioimmunoassay (RIA) and stable isotope dilution assays involving gas chromatography–mass spectrometry (GC/MS). Bioassays have the advantage of actually measuring biological activity and have been of critical qualitative importance in the discovery of prostaglandins. Because of the evanescence of both PGI₁ and TXA₂ in the circulation, RIAs and GC/MS methods are directed toward metabolites. These methods are more sensitive and specific than bioassay and more applicable to quantitative study of prostaglandin turnover in man. Initially, the various approaches appeared to give similar results. PGI₁, unlike other prostaglandins, is not extensively metabolized by the lung, and experiments using bioassay suggested that production by the lung and perhaps other sources permitted PGI₁ to function as a circulating platelet-inhibitory hormone in rabbits and cats. Apparent confirmation of this observation in man was soon provided by transpulmonary measurements of 6-keto-PGF₁α by GC/MS in patients undergoing cardiac catheterization. The inactive hydration product of PGI₁, 6-keto-PGF₁α, is formed nonenzymatically and is more stable than the parent compound. This study suggested that the pulmonary production rate of PGI₁ was in the order of 5 ng/kg/min in man, higher than the threshold dose required to inhibit platelet aggregation ex vivo when synthetic PGI₁ was infused into human volunteers.

Radioimmunoassays for TXB₂, the inactive hydration product of TXA₂, were developed and applied to measurements in human plasma, and many reports characterized a supposed "imbalance of the PGI₁/TXA₂ ratio" in a variety of human diseases. However, in the past 2 years, evidence has appeared that the secretion rate of endogenous PGI₁ into the circulation of human volunteers is extremely low (< 0.09 ng/kg/min). That 6-keto-PGF₁α in human plasma obtained under resting conditions is in the low picogram range (< 5 pg), and that pulmonary release of PGI₁ is undetectable in healthy volunteers. Thus, although PGI₁ is probably important in local platelet-vascular interactions, it does not function as a circulating hormone under physiologic conditions in man. Similarly, we have found that immunoreactive TXB₂ is undetectable in human plasma obtained from healthy volunteers when care was taken to avoid artifacts induced by sampling and technically acceptable assay methods with limits of sensitivity in the low picogram range were combined with highly specific antibodies.

The importance of these observations is that they invalidate the majority of studies pertaining to endogenous biosynthesis of PGI₁ and TXA₂ in human disease. Studies of PGI₁ and TXA₂ production in the human coronary circulation illustrate this point. Samples are usually obtained after insertion of catheters, a procedure that is likely to traumatically stimulate vascular PG₁₁ synthesis. Other aspects of the procedure, such as the use of heparin and radiocontrast media, may also alter synthesis of either compound. Platelet activation at the catheter tip and ex vivo prostanoic formation represent other sources of potential artifact. Finally, it may not be apparent that levels of these compounds are artifically elevated, because the data are often presented as 6-keto-PGF₁α/TXB₂ ratios or coronary sinus/aortic blood ratios of either compound.

It has been proposed that substantial quantitative differences between bioassay estimates of prostacyclin-like activity (2–6 ng/ml) and measurement of 6-keto-PGF₁α in plasma by negative ion chemical ioniza-
tion GC/MS (0.5–2 pg/ml)\(^{18}\) may result from predominant conversion of PGI\(_2\) to 6-keto-PGE\(_{1\alpha}\),\(^{20}\) a biologically active compound\(^{21, 22}\) that is a putative metabolite of PGI\(_2\) in vivo.\(^{23}\) However, this seems highly unlikely because studies of the metabolic disposition of PGI\(_2\) in man have failed to detect metabolites with the 6-keto-PGE structure\(^{24}\) and because infusion of synthetic PGI\(_2\) in volunteers does not result in accumulation of 6-keto-PGE\(_1\) in plasma.\(^{25}\)

One method of circumventing problems of sampling-induced artifact and ex vivo prostaglandin formation is by measurement of metabolites that have an extended half-life. Although the 13,14-dihydro-6,15-diketo-PGF\(_{1\alpha}\) metabolite of PGI\(_2\) has a longer half-life than 6-keto-PGF\(_{1\alpha}\) and has been identified in human plasma after PGI\(_2\) infusions,\(^{26}\) experience with other prostaglandins has shown that the metabolites initially formed from the hydration products are rapidly replaced by more polar products as the major compounds in blood. Measurement of tetraneur metabolites has been recently\(^{27}\) utilized to detect phasic release of PGF\(_{1\alpha}\) in patients with primary dysmenorrhea who suffer episodic pain. Quantitative assays for stable oxidative metabolites of PGI\(_2\) and TXA\(_2\) in plasma are currently being developed.

An alternative approach has been the measurement of prostaglandin breakdown products in urine. When this is performed by GC/MS, it represents a highly specific, noninvasive index of endogenous prostaglandin biosynthesis. Measurement of urinary metabolites by RIA is even more liable to problems of sensitivity and specificity than in plasma. Performance of RIAs on unextracted urine and the use of antibodies with high cross-reactivity to structurally related compounds have been common sources of error. However, the combination of rigorous sample purification with highly specific antibodies has permitted the measurement of 6-keto-PGF\(_{1\alpha}\)\(^{16, 28}\) and TXB\(_2\)\(^{20}\) in urine by RIA. Although 6-keto-PGF\(_{1\alpha}\)\(^{24}\) and TXB\(_2\)\(^{30}\) are urinary metabolites of infused PGI\(_2\) and TXB\(_2\), in man, these indexes are likely to predominantly reflect renal PGI\(_2\) and TXA\(_2\) generation under physiologic conditions.\(^{16, 31}\) Should extrarenal production of either compound be increased (e.g., during a vascular catastrophe), one would anticipate an increase in the urinary levels of the relevant hydration product. For example, urinary 6-keto-PGF\(_{1\alpha}\) excretion is increased in preterm infants with persistent ductus arteriosus and respiratory distress syndrome in whom an increase in systemic prostaglandin biosynthesis is likely.\(^{32}\) The dinor metabolites of 6-keto-PGF\(_{1\alpha}\) and TXB\(_2\), are the most abundant products of systemically administered, radiolabeled PGI\(_2\)\(^{24}\) and TXB\(_2\),\(^{30}\) respectively, in man (table 1). Thus, urinary levels of 2,3-dinor-6-keto-PGF\(_{1\alpha}\) (PGI-M) and 2,3-dinor-TXB\(_2\) (TX-M) have been used to quantify the effects of aspirin and similar drugs upon systemic PGI\(_2\) and TXA\(_2\) synthesis in man\(^{33-35}\) and to accurately predict plasma levels of endogenous PGI\(_2\) in the low picogram range.\(^{13}\)

Although these measurements represent the only noninvasive method of quantifying endogenous generation of PGI\(_2\) and TXA\(_2\), they are not specific to the tissue or origin of these compounds. More specific indexes have been studied ex vivo, such as TXB\(_2\) formation in serum\(^{28, 30}\) or in stimulated platelet-rich plasma\(^{19}\) or 6-keto-PGF\(_{1\alpha}\) formation by biopsies of vascular

### Table 1. Indexes of Endogenous Prostacyclin and Thromboxane A\(_2\) Biosynthesis in Man

<table>
<thead>
<tr>
<th>Biological fluid</th>
<th>Assay method</th>
<th>Notes</th>
</tr>
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<tbody>
<tr>
<td><strong>Prostacyclin:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. 6-keto-PGF(_{1\alpha})</td>
<td>Plasma</td>
<td>GC/MS and RIA</td>
</tr>
<tr>
<td>2. 6,15-diketo-13,14-dihydro-PGF(_{1\alpha})</td>
<td>Plasma</td>
<td>RIA</td>
</tr>
<tr>
<td>3. 6-keto-PGE(_1)</td>
<td>Plasma</td>
<td>GC/MS</td>
</tr>
<tr>
<td>4. 6-keto-PGF(_{1\alpha})</td>
<td>Urine</td>
<td>GC/MS and RIA</td>
</tr>
<tr>
<td>5. 2,3-dinor-6-keto-PGF(_{1\alpha})</td>
<td>Urine</td>
<td>GC/MS</td>
</tr>
<tr>
<td>6. 6,15-diketo-13,14-dihydro-2,3-dinor-PGF(_{1\alpha})</td>
<td>Urine</td>
<td>GC/MS</td>
</tr>
<tr>
<td><strong>Thromboxane A(_2):</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Thromboxane B(_2)</td>
<td>Plasma</td>
<td>RIA</td>
</tr>
<tr>
<td>2. Thromboxane B(_2)</td>
<td>Urine</td>
<td>GC/MS and RIA</td>
</tr>
<tr>
<td>3. 2,3-dinor-thromboxane B(_2)</td>
<td>Urine</td>
<td>GC/MS</td>
</tr>
</tbody>
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Abbreviations: GC/MS = gas chromatography–mass spectrometry; RIA = radioimmunoassay.
tissue.\textsuperscript{37} However, these measurements are related to capacity rather than to actual production rates in vivo. For example, although biopsies of atherosclerotic vessels have a lower capacity to release 6-keto-PGF\textsubscript{1α} than do those of healthy vessels ex vivo,\textsuperscript{38} endogenous PGI\textsubscript{2} biosynthesis is actually increased in patients with diffuse atherosclerosis and platelet activation, perhaps resulting from traumatic platelet-vascular interactions in vivo.\textsuperscript{39}

In conclusion, measurement of urinary metabolites represents the only noninvasive approach to quantitation of endogenous PGI\textsubscript{2} and TXA\textsubscript{2} biosynthesis. Methodologic difficulties and sampling artifacts have cast serious doubt on much of the published data, which are dependent on plasma measurements of TXB\textsubscript{2} and 6-keto-PGF\textsubscript{1α}. The relationship of tissue-specific, capacity-related indexes to endogenous production rates of PGI\textsubscript{2} and TXA\textsubscript{2} under conditions of stimulation and depression is largely unknown. Clarification of the importance of PGI\textsubscript{2} and TXA\textsubscript{2} in human cardiovascular disease awaits the more widespread application of reliable analytical techniques in this area.

References


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