Stimulated Tissue Plasminogen Activator Release as a Marker of Endothelial Function in Humans

James J. Oliver, David J. Webb, David E. Newby

Abstract—The initiation, modulation, and resolution of thrombus associated with eroded or unstable coronary plaques are critically dependent on the efficacy of endogenous fibrinolysis. This is dependent on the cellular function of the surrounding endothelium and vascular wall. In particular, the acute release of tissue plasminogen activator from the endothelium makes an important contribution to the defense against intravascular thrombosis. Here, we describe the rationale and methodology for, and clinical relevance of, assessing acute endothelial tissue plasminogen activator release in humans. The investigation of endothelial fibrinolytic function has the potential to provide major new insights into the pathophysiology of cardiovascular disease, and to shape future therapeutic interventions. (Arterioscler Thromb Vasc Biol. 2005;25:2470-2479.)

Key Words: arterial thrombosis ■ endothelial function ■ endothelium ■ fibrinolysis ■ thrombosis

The endothelium plays a vital role in the control of blood flow, coagulation, fibrinolysis, and inflammation. To date, clinical studies have focused on the assessment of endothelium-dependent vasomotion as a surrogate measure of endothelial function, and there is now extensive evidence of abnormal endothelium-dependent vasodilatation in patients with atherosclerosis and its associated risk factors.1 Abnormal vasomotor responses independently predict cardiovascular events.2 However, endothelium-dependent vasomotion may not be representative of other important aspects of endothelial function, such as the regulation of fibrinolysis.

In health, the endothelium prevents thrombus formation through a number of mechanisms. Thrombomodulin, protein S, heparan sulfate proteoglycans, and tissue factor pathway inhibitor are all endothelium-derived inhibitors of coagulation, whereas prostacyclin, nitric oxide (NO), and surface-bound CD39 inhibit platelet aggregation. However, when endothelial function is perturbed, for example with injury or inflammation, it can rapidly become procoagulant by downregulating its anticoagulant functions, inducing tissue factor expression and increasing secretion of factors such as fibronectin, von Willebrand factor (vWF), and platelet activating factor.3

Original received June 16, 2005; final version accepted September 23, 2005.
From Centre for Cardiovascular Science (J.J.O., D.J.W., D.E.N.), University of Edinburgh, Edinburgh, UK.
Correspondence to Dr James J. Oliver, Clinical Pharmacology Unit and Research Centre, University of Edinburgh, Western General Hospital, Crewe Rd S, Edinburgh, EH4 2XU. E-mail James.Oliver@ed.ac.uk
© 2005 American Heart Association, Inc.

Arterioscler Thromb Vasc Biol. is available at http://www.atvbaha.org

DOI: 10.1161/01.ATV.0000189309.05924.88

2470
Endogenous Fibrinolysis

The endogenous fibrinolytic system protects the circulation from intravascular fibrin formation and thrombosis. After initiation of thrombus formation, the endothelium acutely releases tissue plasminogen activator (t-PA) in response to a range of factors predominantly related to the coagulation cascade, especially factor Xa and thrombin.4 Once released, t-PA catalyzes the conversion of plasminogen to plasmin, facilitating thrombus dissolution through the proteolytic degradation of fibrin to soluble fibrin degradation products (Figure 1). The conversion of plasminogen to plasmin by t-PA is accelerated in the presence of fibrin and at the endothelial cell surface,5,6 ensuring efficient localized activation. Because plasminogen is present at vast molar excess over t-PA in plasma, the onset and efficacy of fibrinolysis are principally determined by the rapidity and magnitude of t-PA release.

The concentration of t-PA in human plasma is \( \approx 3 \) to 10 ng/mL, but a relatively small proportion is functionally active because of the presence of serine protease inhibitors (serpins); principally, plasminogen activator inhibitor (PAI) type 1 (PAI-1), but also PAI-2, PAI-3, \( \alpha_2 \)-macroglobulin, and C1 esterase inhibitor (Figure 2).7 The proportion of active t-PA varies inversely with plasma PAI-1 concentration, from 2% to 33%.8 The plasma half-life of t-PA is \( \approx 5 \) minutes, and the liver is the major site of clearance.8 The interaction between t-PA and PAI-1 has a rapid second order rate constant of \( \approx 10^7 \) M\(^{-1}\) s\(^{-1}\),10 and there is a several-fold molar excess of PAI-1 over t-PA in plasma.8 Therefore, for active unbound t-PA to reach a thrombus, rapid local release is vital, particularly because fibrinolysis is much more effective if t-PA is incorporated during, rather than after, thrombus formation.11

Synthesis, Storage, and Release of t-PA

Encoded by a gene on chromosome 8, t-PA is a 68 kDa serine protease of 530 amino acids and the endothelium is its principal site of generation. Endothelial cells in culture synthesize and constitutively secrete t-PA.12 The rate of synthesis is increased by a number of substances, including thrombin and histamine,13 and is reduced by plasmin.14 However, although protein kinase C appears to play a role,15 the mechanisms regulating t-PA synthesis have not been characterized in detail.

t-PA is released facultatively from storage granules and the pathways of constitutive and facultative release differ.12 Some workers have suggested that t-PA is stored with vWF in Weibel-Palade bodies,16 but there is now convincing evidence that t-PA is stored in vesicles distinct from Weibel-Palade bodies,17,18 and this is consistent with the in vivo observation

![Figure 1](http://atvb.ahajournals.org/)

**Figure 1.** The endothelial fibrinolytic response to luminal thrombus. Agonists generated from the coagulation cascade act on endothelial cell surface G protein-coupled receptors (GPCRs) (1) to stimulate release of tissue plasminogen activator (t-PA) from storage granules, a step that requires an increase in intracellular calcium (Ca\(^{2+} \)) concentration (2). Free t-PA acts on thrombus-bound plasminogen (3) to produce plasmin (4) that, in turn, degrades cross-linked fibrin into fibrin degradation products (FDPs) (5), thus dissolving the thrombus. The fibrinolytic process is inhibited by inactivation of t-PA by PAI-1 and plasmin by \( \alpha_2 \)-antiplasmin.

![Figure 2](http://atvb.ahajournals.org/)

**Figure 2.** Relationship between tissue plasminogen activator (t-PA) and its major inhibitor, plasminogen activator inhibitor type 1 (PAI-1), in plasma. Only the unbound fractions are active. Other protease inhibitors, including PAI-2, PAI-3, \( \alpha_2 \)-macroglobulin, and C1 esterase inhibitor, also bind to t-PA in plasma.
Substances That Stimulate Acute Tissue Plasminogen Activator (t-PA) Release in Humans

<table>
<thead>
<tr>
<th>Substance</th>
<th>Mechanism</th>
<th>Dose</th>
<th>Comments</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bradykinin</td>
<td>B&lt;sub&gt;2&lt;/sub&gt; receptor-dependent</td>
<td>0.02 to 3 nmol/min</td>
<td>Bradykinin-induced t-PA release potentially relevant to contact phase of the intrinsic coagulation pathway and the action of ACE inhibitors</td>
<td>20, 28, 40, 42, 51, 52, 54, 55, 57, 91, 93, 98, 99</td>
</tr>
<tr>
<td>Substance P</td>
<td>NK&lt;sub&gt;1&lt;/sub&gt; receptors</td>
<td>2 to 40 pmol/min</td>
<td>t-PA release has delayed onset and is sustained for ≥3 hours after infusion is stopped. May induce de novo t-PA protein synthesis.</td>
<td>43, 53</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Uncertain</td>
<td>80 to 240 ng/min</td>
<td></td>
<td>73</td>
</tr>
<tr>
<td>Desmopressin</td>
<td>V&lt;sub&gt;2&lt;/sub&gt; receptors</td>
<td>21 to 70 ng/min</td>
<td></td>
<td>26, 33, 48, 60, 95</td>
</tr>
<tr>
<td>Isoprenaline</td>
<td>Adrenergic receptors</td>
<td>400 ng/min</td>
<td></td>
<td>47</td>
</tr>
<tr>
<td>ATP</td>
<td>P2X or P2Y receptors</td>
<td>10 to 200 nmol/min</td>
<td></td>
<td>67</td>
</tr>
<tr>
<td>UTP</td>
<td>P2Y receptors</td>
<td>0.8 to 12.8 μg/min</td>
<td></td>
<td>26, 33, 46, 47, 62, 93</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>Adrenergic receptors</td>
<td>1.2 μg/min</td>
<td></td>
<td>41</td>
</tr>
</tbody>
</table>

Acute release results from translocation of a dynamic intracellular storage pool<sup>12</sup> in response to blood coagulation and humoral factors.<sup>4</sup>

In Vivo Assessment

In humans, acute release of t-PA can be assessed systemically, for example after intravenous infusion of desmopressin<sup>31</sup> or bradykinin.<sup>32</sup> However, this approach is limited by potential confounding effects, such as changes in systemic hemodynamics, clearance of t-PA and PAI-1, activation of the sympathetic nervous system, and concomitant release of other mediators. Direct assessment of local capacity for acute t-PA release within individual vascular beds avoids these problems and is likely to better represent the defense against arterial thrombosis. Local availability of active t-PA depends on the extent of local t-PA release rather than on the amount of t-PA or PAI-1 entering the tissue in arterial blood.<sup>33</sup>

Venous Occlusion Test

Regional t-PA release can be assessed in vivo by measuring the increase in t-PA concentration in pooled venous plasma. Typically, an upper arm or leg cuff is inflated to between systolic and diastolic blood pressure to cause venous pooling. Blood is sampled before and 10 or 20 minutes after cuff inflation.<sup>34</sup> Using this methodology, reduced t-PA release has been reported in various groups of subjects, including those who smoke,<sup>35</sup> although there are conflicting data on patients with coronary artery disease.<sup>36,37</sup> Whereas the increase in t-PA in pooled venous blood may simply be because of continued secretion at the basal rate,<sup>25</sup> it has been suggested that venous occlusion itself stimulates t-PA release.<sup>38</sup> Although quite simple and widely applicable, the venous occlusion technique is a rather blunt tool with relatively poor reproducibility.<sup>39</sup>

Regional Tissue Release

Acute t-PA release has been assessed in both the forearm and coronary circulations of humans using a number of endothelial stimulants including bradykinin, substance P, desmopressin, and methacholine (Table).
Forearm Release

Two similar methodologies are used to assess forearm t-PA release in response to intrabrachial infusions: one based on the differences in plasma concentrations of t-PA between inflowing arterial and outflowing venous plasma of a single arm,40–42 and the other based on the differences in venous plasma concentrations between the 2 arms.43 For the arteriovenous technique, drugs are infused via an 18-gauge catheter and the arteriovenous concentration gradient is calculated from blood samples taken simultaneously from this and an ipsilateral venous catheter. Forearm plasma flow is calculated using forearm blood flow (FBF), measured by strain gauge plethysmography, and arterial hematocrit corrected for 1% trapped plasma. Net release is calculated as the product of the arteriovenous concentration gradient and forearm plasma flow:

Net release = \((C_v - C_a) \times FBF \times \frac{(101 - \text{hematocrit})}{100}\)

where \(C_v\) and \(C_a\) are the venous and arterial concentrations, respectively. The antigen concentrations of t-PA are measured using enzyme-linked immunosorbent assays,44 and net release is expressed as ng per 100 mL of forearm tissue per minute. The activity of t-PA is determined photometrically45 and expressed as IU per 100 mL of forearm tissue per min.

Typical resting arteriovenous differences in t-PA concentrations in the forearm are only \(\approx 10\%\) of total venous concentration and basal constitutive release is \(\approx 0.4\) to 1.3 ng/100 mL of tissue/min.19,26,40,42,46–48 There is no demonstrable release of PAI-1 across the forearm; therefore, t-PA activity increases in parallel with t-PA antigen concentrations.19,46–48 There is no consensus on whether it is better to measure t-PA antigen or activity. Whereas it is only unbound t-PA that is functionally active (Figure 2), ultimately the efficacy of endogenous fibrinolysis is determined by the magnitude of local t-PA release and the resultant t-PA activity at the site of thrombus. Whether venous plasma t-PA antigen or activity concentrations best reflect this dynamic process remains unclear and will in part depend on local tissue perfusion rates and plasma serpin concentrations. Consequently, both t-PA antigen and activity are often presented.

Using the venovenous technique, drugs are infused into the brachial artery via a 27-gauge needle. This is narrower than the catheter used in arteriovenous studies because it is not used for blood sampling. Net release is calculated in a similar manner, except that the concentration gradient is that between the venous plasma of the infused and noninfused arms. The reproducibility of agonist-induced t-PA release using the venovenous technique has been shown to be good.49

The arteriovenous and the venovenous approaches both have benefits and limitations. Net release is calculated directly in the arteriovenous technique but indirectly in the venovenous technique, potentially resulting in different estimates of stimulated t-PA release under certain conditions (Figure 3). Basal release cannot be calculated using the venovenous technique. However, because basal release constitutes only a small proportion of the overall venous plasma concentration, the venovenous technique does provide an accurate assessment of stimulated t-PA release. The arteriovenous method is technically more challenging and the larger-bore arterial catheter makes it less suitable for repeated studies within individuals. Moreover, the arterial catheter presents a larger thrombogenic surface that may itself stimulate the fibrinolytic system. Both techniques assume that there is no clearance of t-PA across the forearm. This is a reasonable assumption because the liver is the major site of clearance, although there are endothelial receptors capable of clearing t-PA from the circulation.50

Cardiac Release

In the coronary circulation, dynamic t-PA release is assessed by infusion of agents into the left main51,52 or left anterior descending coronary artery,53 and samples are obtained simultaneously from the coronary sinus and either the aorta or femoral artery.51–54 Sampling blood from the coronary sinus is appropriate only during the evaluation of the left ventricle and, particularly, during left anterior descending artery infusions. Care must be taken to ensure that catheters do not impede flow in the coronary sinus, because this may divert blood into the anterior cardiac or Thebesian veins. Net t-PA release is calculated in a similar manner to the forearm technique, but with blood flow measured using coronary
Doppler combined with either quantitative coronary angio-
graphy or intravascular ultrasound (IVUS).

**Stimulation of t-PA Release**

Although a number of humoral and coagulant factors cause t-PA release, other factors, including angiotensin II, acetyl-
choline, and atrial natriuretic peptide, do not release t-PA, despite causing marked vasoconstrictor effects.

Bradykinin is an inflammatory vasodilator peptide and a powerful stimulant of endothelial t-PA release. It acts via the B1 receptor in an NO-independent and prostaglandin (PG)-
independent fashion. An endothelium-derived hyperpolarizing factor probably contributes to bradykinin-induced va-
sodilatation, although whether endothelium-derived hyperpolarizing factor contributes to t-PA release is not known.

The tachykinin vasodilator, substance P, acts mainly through the neurokinin type 1 (NK1) receptor. It is a central and peripheral neurotransmitter and mediates neurogenic inflammation. Substance P induces t-PA release in both the forearm and coronary circulations, and is the most potent known stimulant of t-PA in humans.

The vasopressin analogue, desmopressin, is a V2 receptor agonist that releases t-PA in the forearm circulation. When administered systemically, it also induces vWF release.

The muscarinic receptor agonist methacholine induces t-PA release in the forearm, but to a lesser degree than bradykinin or desmopressin. In contrast, acetylcholine, also a muscarinic agonist, does not stimulate t-PA release. The reason for this difference is not clear, although varying potencies and stabilities of the respective compounds may be responsible. These agents also appear to provoke vasodilatation via differing mechanisms with acetyl-
choline, but not methacholine, being largely mediated through NO production.

The adrenergic agonists, norepinephrine and isopro-
ephrine, both induce t-PA release in the forearm, as does mental stress, which is associated with adrenergic activation.

These data suggest a role for the sympathetic nervous system in controlling vascular t-PA secretion. In support of this, chemical sympathectomy in rats reduces basal plasma t-PA concentrations and agonist-induced t-PA release in isolated vessels. In a porcine model, stimulation of cardiac sympa-
thetic nerves causes coronary t-PA release. Although norepinephrine from sympathetic nerve endings may act on the endothelium to release t-PA, there is also intriguing evidence that sympathetic neurons can themselves synthesize and release t-PA. Both adenosine and uridine triphosphates (ATP and UTP) stimulate t-PA release in the forearm, apparently indepen-
dently of NO and PG pathways. This is of particular interest because these nucleotides are released by activated platelets, endothelial cells and cardiomyocytes during ischemia. The doses of ATP and UTP administered resulted in similar plasma concentrations of the nucleotides to those commonly observed in the context of ischemia. Moreover, coronary ischemia in the pig was associated with increased t-PA release.

**Regulation of t-PA Release**

The role of NO in the mechanism of endothelial t-PA release is unclear. Sodium nitroprusside, a spontaneous NO donor, does not stimulate either synthesis or release in cultured endothelial cells or stimulate release in vivo. However, substance P-induced t-PA release was inhibited by the NO synthase inhibitor, N\textsubscript{\textgreek{g}} monomethyl-L-arginine (L-
NMMA). In contrast, bradykinin-induced t-PA release was unaffected by L-NMMA in one study and increased by L-NMMA in another. Thus, NO alone does not induce t-PA release, although it may play a permissive or synergistic role in stimulated t-PA release.

Shear stress is a well-characterized stimulus for t-PA secretion from cultured endothelial cells. In ex vivo human conduit vessels, shear stress stimulates t-PA expression and increases its intracellular storage pool without stimulating its release. Consistent with this, marked increases in blood flow during infusions of vasodilators such as sodium nitro-
prusside and papaverine do not cause in vivo t-PA release. A permissive role of increased shear stress is also unlikely given that intrabrachial tumor necrosis factor-α induces t-PA release without changing blood flow, and the vasoconstrictor, norepinephrine, releases t-PA while reducing flow. Thus, blood flow and shear stress appear to regulate endothelial t-PA synthesis and storage but do not affect its acute release.

**Genetic Influences on t-PA Release**

A number of small studies have investigated genetic influ-
ences on dynamic endothelial t-PA release. An Alu-repeat polymorphism and 3 single nucleotide polymorphisms in linkage disequilibrium with this associate with t-PA release rates. Although not apparent in the forearm circulation, angiotensin-converting enzyme (ACE) I/D genotype may influence bradykinin-induced t-PA release in the coronary circulation. In addition, there was no difference between healthy black and white Americans in bradykinin-induced t-PA release. Further studies, with larger sample sizes, are desirable to confirm genetic linkage.

**Clinical Relevance of Endothelial t-PA Release**

Acute rupture or erosion of a coronary atheromatous plaque, and subsequent thrombosis, cause the majority of sudden cardiac deaths and myocardial infarctions. Small areas of denudation and thrombus are commonly found on atheroma-
tous plaques and are usually subclinical. However, with imbalance in the fibrinolytic system, such microthrombi may propagate, leading to arterial occlusion. The importance of acute endogenous t-PA release is further exemplified by the high rate of spontaneous reperfusion in the infarct-related artery after acute myocardial infarction. A reduction in t-PA activity or release is associated with an increased incidence of major adverse cardiac events in patients with stable or unstable angina. Rosenberg and Aird have postulated that vascular bed-specific defects in hemostasis exist, and that coronary thrombosis critically depends on local fibrinolytic balance.
Basal Plasma t-PA Concentrations
Several studies have investigated the relationship between basal venous t-PA antigen concentrations and subsequent coronary heart disease. In a meta-analysis of prospective studies, the risk of coronary heart disease was \( \approx 50\% \) greater in those with plasma t-PA antigen concentrations in the highest tertile compared with those in the lowest tertile. This may seem counterintuitive but in part reflects the concomitant increase of plasma PAI-1 concentrations and associated reduction in t-PA activity. However, whereas this epidemiology is of interest, basal plasma t-PA does not reflect the local capacity for acute endothelial t-PA release in response to developing thrombus. This underscores the importance of assessing acute endothelial t-PA release that is likely to be of greater pathophysiological relevance.

Acute Coronary t-PA Release
The acute fibrinolytic activity of the heart is inversely correlated with the extent of proximal coronary artery atherosclerosis. The mechanisms underlying this relationship are likely to involve chronic endothelial cell injury and impaired vascular function. Alternatively, this association may reflect chronic stimulation and upregulation of basal t-PA release secondary to atheroma and arterial denudation. The subsequent depletion of endothelial t-PA stores, and the desensitization and reduction of the acute fibrinolytic response, would potentially be detrimental.

Questions of cause and effect cannot be resolved by such observations and it remains possible that reduced fibrinolytic activity enhances atherogenesis. The prolonged presence of residual thrombus over a disrupted plaque will provoke smooth muscle migration and new connective tissue production, leading to plaque expansion. This is consistent with the enhanced macrovascular fibrin deposition and atherogenesis seen in genetic murine models of t-PA deficiency.

Smoking
Basal plasma t-PA concentrations are either increased or unaltered in chronic smokers, but dynamic endothelial t-PA release is dramatically reduced. This has been consistently demonstrated in the forearm and coronary circulations (Figure 4), as well as with the venous occlusion test and systemic desmopressin infusion. The increased risk of spontaneous thrombosis in smokers may, therefore, relate to propagation of thrombus, which would otherwise undergo lysis and remain subclinical.

Although smokers have a higher overall mortality from myocardial infarction than nonsmokers, their in-hospital mortality is lower (Figure 4). This may, in part, be explained by the finding that in current smokers the infarct related artery is more likely to become patent after thrombolytic therapy. These observations are consistent with these findings on endothelial t-PA release because it might be anticipated that patients with impaired endothelial cell t-PA release would benefit most from thrombolytic therapy, whereas those with a normal endogenous fibrinolytic capacity are more likely to have coronary thrombus resistant to fibrinolysis.

Hypercholesterolemia
Patients with hypercholesterolemia have impaired endothelial-dependent vasodilation. However, in contrast to smokers, hypercholesterolemia and lipid-lowering therapy do not influence acute t-PA release. This is consistent with the finding that serum cholesterol concentrations, unlike smoking status, do not influence the patency rate of the infarct related artery after thrombolytic therapy. Moreover, hypercholesterolemia is particularly associated with vulnerable plaque rupture, whereas acute thrombosis develops in smokers even without plaque rupture. It would therefore appear that, in contrast to smoking, hypercholesterolemia-associated plaque rupture is such a dramatic event that thrombosis occurs despite preserved local t-PA release.

Hypertension
Marked impairment of desmopressin-induced t-PA release but not vasodilatation has been demonstrated in hypertensives, perhaps suggesting that impaired t-PA release may be a more sensitive marker of endothelial damage in hypertension. However, in contrast to the impaired desmopressin response, methacholine-induced t-PA release is unaffected. Similar agonist-specific defects have been reported in smokers, in whom bradykinin and substance P but not methacholine, induced t-PA release is impaired. Although the explanation for this discrepancy is unclear, it may simply be more difficult to detect differences in release with a relatively weak stimulus, such as methacholine. Alternatively, hypertension and smoking may have different effects.
Bradykinin and ACE Inhibition

Bradykinin is a potent endothelium-dependent vasodilator that has a brief duration of action caused by its rapid degradation by ACE. In addition to being an inflammatory mediator, it is closely involved in the fibrinolytic and coagulation cascades. During the contact phase of blood coagulation, it is released after the cleavage of high-molecular-weight kininogen by kallikrein and is a potent stimulant for endothelial t-PA release. Thus, when plaque rupture or erosion activates the intrinsic coagulation pathway, liberation of bradykinin may provide important negative feedback to limit thrombus development. In keeping with this, bradykinin generation is transiently increased after an episode of unstable angina.

Potentiation of endothelial t-PA release through the preservation of endogenous bradykinin might account for some of the cardiovascular benefits of ACE inhibitors. In support of this, in the forearm of healthy men and patients with heart failure, ACE inhibition augments bradykinin, but not substance P, induced t-PA release. Similarly, augmentation of bradykinin-induced t-PA release by ACE inhibition has been demonstrated in the coronary circulation. The effect of ACE inhibition on t-PA release is mediated specifically through bradykinin, because angiotensin II receptor blockade has no effect on bradykinin-induced t-PA release.

Bradykinin antagonism reduces local t-PA release during intra-brachial enalaprilat infusion, providing direct evidence that ACE inhibition augments endothelial t-PA release through increased endogenous bradykinin. ACE inhibition might also enhance endothelial t-PA release by increasing bradykinin receptor expression.

Endothelial t-PA Release as a Novel Measure of Endothelial Function

The endothelium has a number of important functions, including regulation of vascular tone, coagulation, fibrinolysis, and inflammation. However, to date, most clinical studies on endothelial function have focused on endothelium-dependent vasomotion. Although this is a useful surrogate marker for the role of the endothelium in atherothrombosis, measuring other aspects of endothelial function, including the capacity for t-PA release, may provide additional and novel insights.

Reports of preserved endothelium-dependent vasodilatation in smokers and in patients with hypertension despite reduced acute t-PA release suggest that, in some circumstances, reduced t-PA release may be a more sensitive marker of endothelial dysfunction. Moreover, some conditions associated with impaired endothelium-dependent vasodilatation, such as hypercholesterolemia, are not accompanied by reduced t-PA release. These data highlight the complexity of vascular biology, and that it is perhaps naive to expect endothelial dysfunction to be expressed by a uniform phenotype irrespective of the insult. Vascular inflammation is associated with augmentation of t-PA release despite impairing endothelium-dependent vasodilatation. This calls into question the use of the term endothelial dysfunction. Is the cell truly dysfunctional or in an altered state of activation? In particular, the dominant use of this term to refer to endothelium-dependent and NO-dependent vasodilatation seems unnecessarily restrictive and inaccurate. The many and varied actions of the endothelium may be potentiated and inhibited simultaneously within the same cell. Whether this is ultimately beneficial or detrimental depends on the local and systemic setting of the individual vessel, tissue, and patient.

Limitations of Clinical In Vivo Models

To date, most studies of dynamic endothelial t-PA release have been relatively small because of the invasive nature of the techniques. Measurement of coronary t-PA release is likely to be of greatest relevance to coronary pathophysiology but can only be performed in selected subjects undergoing coronary angiography. Whereas forearm assessment can be performed more widely, this vascular bed is less susceptible to atherosclerosis and subsequent thrombosis, raising questions on its validity as a surrogate for the coronary circulation. Nevertheless, consistent findings between the peripheral and coronary circulations support the notion that the forearm model is a reasonable surrogate.

Although quantitative assessment of endothelial t-PA release can be made using local infusions, the clinical models used are relatively simplistic when compared with the complex in vivo response to developing thrombus. For example, measuring t-PA release in response to components of the coagulation cascade, such as activated factor X or thrombin, might more closely mirror in vivo pathophysiology but would risk inducing arterial thrombosis. The in vivo clinical models also measure t-PA release across an entire vascular bed and it does not necessarily follow that such measures reflect the local capacity for t-PA release at the site of an eroded or ruptured plaque.

Future Work

It can be reasonably hypothesized that reduced dynamic endothelial t-PA release predisposes to events such as myocardial infarction and unstable angina. However, longitudinal studies of t-PA release as a predictor of coronary events have not been performed. Whereas assessment of t-PA release is unlikely to become part of cardiovascular risk assessment, demonstrating that impaired release predicts cardiovascular events would support the hypothesis that reduced t-PA release is of pathophysiological significance.

Although the capacity for endothelial t-PA release is clearly reduced in some circumstances, the mechanisms are unknown. Serum from smokers, but not from nonsmokers, reduced substance P-induced t-PA release from cultured endothelial cells, suggesting that a blood-borne mediator may affect endothelial t-PA responses. Using ex vivo human umbilical veins, increased intraluminal pressure decreased t-PA release as well as gene and protein expression, suggesting that raised intraluminal pressure might itself impair t-PA release. Detailed characterization of the path-
ways of t-PA secretion and how these are affected by different stimuli will provide a platform for the investigation of the mechanisms of impaired secretion.

Improving t-PA release may represent a new therapeutic target in cardiovascular prevention. There is mounting evidence that some of the benefits of ACE inhibitors may be the result of enhanced t-PA release, whereas statins exert their beneficial effects through alternative pathways. Once the mechanisms of impaired t-PA release have been defined further, there is the potential to develop agents that will specifically augment endothelial t-PA release.

Conclusions

The dynamic regulation of intravascular thrombus formation is central to our understanding of acute and chronic atherosclerotic events. The initiation, modulation, and resolution of thrombus associated with eroded or unstable coronary plaques are critically dependent on the efficacy of endogenous fibrinolysis. It is perhaps remarkable that this fundamentally important aspect of endothelial function has been relatively underinvestigated in clinical studies to date. Here, we have described the various methods of assessing acute endothelial t-PA release in humans and have explored the effects of various cardiovascular conditions and risk factors on the endogenous fibrinolytic capacity. Impaired endothelial t-PA release appears to be a particular feature of cigarette smoking and atherosclerosis but not hypercholesterolemia. Thus, endothelial dysfunction can be manifest in separate distinct pathways depending on the nature of the insult, underscoring the importance of critically investigating the specific consequences of vascular injury. The investigation of endothelial fibrinolytic function has the potential to provide major new insights into the pathophysiology of cardiovascular disease and to shape future therapeutic interventions.

Acknowledgments

The authors are grateful for support from the British Heart Foundation (BHF RG/05/003, BHF PG/04/131, BHF PG/03/017, BHF PG/03/009, BHF PG/02/113) for their research on endothelial fibrinolytic function.

References


Stimulated Tissue Plasminogen Activator Release as a Marker of Endothelial Function in Humans

James J. Oliver, David J. Webb and David E. Newby

Arterioscler Thromb Vasc Biol. 2005;25:2470-2479; originally published online October 6, 2005;
doi: 10.1161/01.ATV.0000189309.05924.88

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2005 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/25/12/2470

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/