# IMPAIRED ENDOGENOUS FIBRINOLYSIS: A MAJOR STIMULUS FOR CORONARY THROMBOSIS AND ATHEROGENESIS*

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**SUMMARY**

Acute myocardial infarction is predominantly caused by thrombotic occlusion of a coronary artery at the site of a ruptured or eroded atheromatous plaque. Local endogenous fibrinolytic activity is a major determinant in the initiation, propagation and resolution of the thrombotic complications of coronary atheroma. This is exemplified by the high rate of spontaneous reperfusion in the infarct-related occluded artery after acute myocardial infarction.

The maintenance and regulation of tissue perfusion critically depends upon the integrity of endothelial function and the release of potent endothelium-derived factors. To date, the assessment of endothelial function has focused on its role in the regulation of vascular tone. Whilst this aspect is important, it represents an indirect measure of the role of the endothelium in athero-thrombosis and does not address the central role of the endothelium in regulating endogenous fibrinolysis through the acute release of tissue plasminogen activator.

Novel models to assess acute tissue plasminogen activator release in the peripheral and coronary circulations have been developed and characterised. Inhibition of nitric oxide synthesis and cigarette smoking are associated with an impairment of acute endothelial tissue plasminogen activator release. This effect may not only explain the increased propensity of smokers to sustain an acute myocardial infarction but also their enhanced response to exogenous thrombolytic therapy and reduced in-hospital mortality when myocardial infarction occurs – the so-called 'smokers' paradox'. Furthermore, the demonstration of an association between the extent of coronary atheroma and the local endogenous fibrinolytic response provides a potentially important mechanism through which endothelial dysfunction can directly contribute to atheroma formation and its associated thrombotic complications.

Studies of endothelial function have now provided the first clinical evidence to support the hypothesis that vascular bed-specific defects in haemostasis exist, and that thrombosis in the coronary circulation critically depends on the local fibrinolytic balance. Moreover, these observations are consistent with a reduced fibrinolytic activity causing enhanced atheroma formation, and are supported by the findings of genetic murine models where atherogenesis is accelerated by fibrinolytic deficiencies.

## ENDOGENOUS FIBRINOLYSIS, ATHEROSCLEROSIS AND THROMBOSIS

Acute rupture or erosion of an atheromatous plaque and subsequent superimposed arterial thrombosis is a major cause of cardiovascular morbidity and mortality. In over three-quarters of cases, the culprit atheromatous plaque is not obstructive and constitutes a luminal stenosis of less than 50%. Small areas of denudation and thrombus deposition are a common finding on the surface of atheromatous plaques but usually remain sub-clinical due to endogenous fibrinolysis and 'passification' of the lesion.[1] However, in the presence of an imbalance in the fibrinolytic system such microthrombi may propagate, ultimately leading to arterial occlusion.[2] Indeed, impairment of the fibrinolytic balance is associated with an increased risk of sudden cardiac death and myocardial infarction.[3]

In addition to having a major impact on the thrombotic consequences of atherosclerosis, endogenous fibrinolysis is also implicated in the atherogenic process itself. Detailed post-mortem studies have shown that plaque growth is induced by episodic subclinical plaque disruption and thrombus formation.[1] The prolonged presence of residual thrombus over a disrupted or eroded plaque will provoke smooth muscle migration, the production of new connective tissue and consequent plaque expansion.[1] Moreover, genetic murine models of fibrinolytic deficiency demonstrate evidence of thrombus-induced myocardial infarction[4] and, in addition, accelerated atherogenesis.[5]

## CIGARETTE SMOKING AND CORONARY THROMBOSIS

Post-mortem studies of patients with sudden cardiac death have demonstrated that dyslipidaemia is associated with plaque rupture whereas cigarette smoking predisposes to acute coronary thrombosis.[6] This thrombotic tendency accounts for the well-described association of smoking habit with coronary artery disease and acute myocardial infarction. Intriguingly, however, cigarette smokers have an in-hospital mortality that is approximately three-times lower than that of non-smokers: the so-called smokers' paradox (see Figure 1).[7] This may in part be explained by the observation that following thrombolytic therapy for

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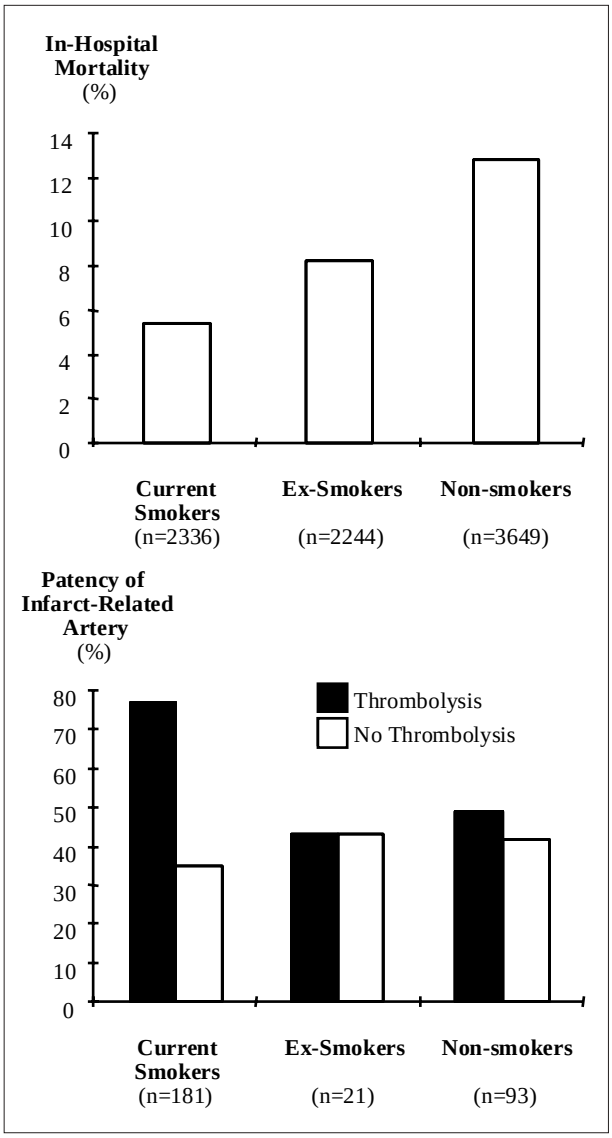



**FIGURE 1**
**The Smokers' Paradox.**
Lower in-hospital mortality and an enhanced reperfusion rate of the infarct related artery with thrombolytic therapy. Data from Barbash *et al.*[7] and De Chillou *et al.*[8]

acute myocardial infarction, the patency rate of the infarct-related artery is markedly enhanced.[8] Indeed, the paucity of the response in non-smokers has lead some workers to suggest that thrombolytic therapy should be reserved only for smokers and alternative strategies, such as primary angioplasty, used in non-smokers. The question that begs an answer is why should cigarette smokers be more sensitive to thrombolytic therapy?

## REGULATION OF ENDOGENOUS FIBRINOLYSIS

The fibrinolytic factor, tissue plasminogen activator (t-PA) is a serine protease that regulates the degradation of intravascular fibrin and is released from the endothelium through the translocation of a dynamic intracellular storage pool. If endogenous fibrinolysis is to be effective, then the rapid mobilisation of t-PA from the endothelium is essential, because thrombus dissolution is much more effective if t-PA is incorporated during, rather than after, thrombus formation.[9] The efficacy of plasminogen activation and fibrin degradation is further determined by the relative balance between the acute local release of t-PA and its subsequent inhibition through formation of complexes with the serpin, plasminogen activator inhibitor type 1 (PAI-1).

The importance of endogenous t-PA release is exemplified by the high rate of spontaneous reperfusion in the infarct-related occluded artery after acute myocardial infarction; this occurs in around one-third of patients within the first 12 hours.[10] The rate and magnitude of acute t-PA release from the endothelium will have major relevance to the pathogenesis of atherothrombosis but, to date, has been underinvestigated due to the lack of appropriate methodology.

## ASSESSMENT OF ACUTE TISSUE PLASMINOGEN ACTIVATOR RELEASE

When studying *in vivo* vascular responses in man, systemic drug administration will cause concomitant effects on other organ systems such as the liver, brain, kidney and heart, as well as influence neuro-humoral reflexes through changes in systemic haemodynamics. Therefore, interpretation of the vascular responses cannot be wholly attributed to a direct effect of the drug on the blood vessels because of these confounding influences. Endogenous fibrinolysis in humans has been assessed using systemic infusion of agents such as desmopressin[11] and angiotensin II.[12] These agents are vasoactive and induce changes in blood pressure and regional blood flow as well as having widespread effects on disparate tissues. Thus, the consequent changes in systemic fibrinolytic parameters will be a combination of many factors, including hepatic production and clearance of t-PA and PAI-1. In contrast, the use of bilateral forearm blood flow measurements with unilateral brachial artery infusion of vasoactive drugs at subsystemic, locally active doses, provides a powerful and reproducible method of directly assessing local vascular responses *in vivo*.[14] Combined with bilateral forearm venous sampling, this technique permits the assessment of local production of tissue factors in the isolated forearm vascular bed with the contralateral non-infused arm acting as a contemporaneous control.

We have recently validated this *in vivo* method of assessing the acute release of t-PA from the peripheral vascular endothelium in humans.[15, 16] Using the neurokinin type 1 receptor-dependent,[17] endothelial stimulant substance P, we have been able to demonstrate a dose-dependent release of t-PA from the forearm without causing the release of other endothelium-derived factors such as PAI-1 and von Willebrand factor (vWf).[15] However, since substance P also causes a marked vasodilatation, this effect could be a direct consequence of the associated increase in forearm blood flow. To address this issue, we assessed

the effect of the endothelium-independent vasodilator, sodium nitroprusside, and were able to demonstrate that equivalent increases in blood flow were not sufficient to cause release of t-PA from the endothelium.[15] This indicates that the release of t-PA is dependent upon direct endothelial cell stimulation rather than changes in the local haemodynamics.

## ENDOTHELIAL FUNCTION AND ACUTE TISSUE PLASMINOGEN ACTIVATOR RELEASE

The endothelium plays a vital role in the control of blood flow, coagulation, fibrinolysis and inflammation. Consequently, the maintenance and regulation of tissue perfusion critically depends upon the integrity of endothelial function and the release of potent endothelium-derived factors.[18] Following the seminal work of Furchgott and Zawadski[19] it has been widely recognised that an array of mediators can influence vascular tone through endothelium-dependent actions, and there is now extensive evidence of abnormal endothelium-dependent vasomotion in patients with atherosclerosis[20] and in those exhibiting its associated risk factors,[21,22] including cigarette smoking.[23,24] However, whilst endothelium-dependent vasomotion is important, it may not be representative for other aspects of endothelial function, such as the regulation of fibrinolysis. Is the acute release of t-PA impaired by endothelial dysfunction?

Through the action of nitric oxide synthase, the endothelium continuously releases nitric oxide to regulate basal vascular tone and blood flow. L-N-monomethyl arginine (L-NMMA) is a selective and competitive inhibitor of nitric oxide synthase and reduces the vasomotor actions of endothelial cell stimulants, such as acetylcholine.[25] However, what effect does nitric oxide synthase inhibition have on the acute release of t-PA from the endothelium? We assessed the actions of substance P and L-NMMA, alone and in combination, on the local forearm release of t-PA.[26] We were able to demonstrate a marked attenuation of substance P induced t-PA release in the presence of L-NMMA.[26] This coupling of acute t-PA release to the L-arginine:nitric oxide pathway provides an important potential mechanism whereby endothelial dysfunction increases the risk of atherothrombosis through a reduction in the acute fibrinolytic capacity. However, is there any evidence to suggest that conditions associated with vascular disease and endothelial dysfunction have an impairment of acute t-PA release?

## IMPAIRED T-PA RELEASE AND CIGARETTE SMOKING

Cigarette smoking is associated with endothelial dysfunction and reduced endothelium-dependent vasodilatation.[23] We applied the forearm model and assessed acute t-PA release in 24 healthy, smoking and non-smoking volunteers. This study demonstrated a marked reduction in substance P induced t-PA release in cigarette smokers.[24]

Impaired endogenous fibrinolysis would, therefore, appear to contribute to the increased risk of coronary thrombosis seen in smokers through propagation of thrombus that would otherwise undergo lysis and remain sub-clinical. Moreover, it provides a potential explanation for the smokers' paradox since it would be anticipated that patients with impaired coronary endothelial cell t-PA release would benefit most from exogenous thrombolytic therapy. Indeed, this is consistent with preliminary evidence in patients with acute myocardial infarction that suggests lower admission plasma t-PA activity is associated with a more rapid response to thrombolytic therapy.[27]

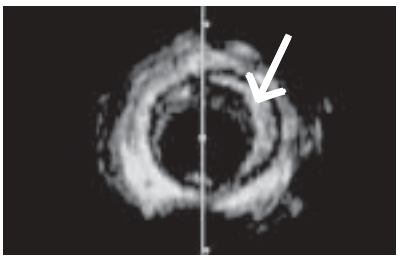
Given that the forearm vascular bed is a privileged site and is usually free of atheroma, what influence does cigarette smoking and atherosclerosis have on the local fibrinolytic activity of the coronary circulation in patients with coronary artery disease?

## CORONARY TISSUE PLASMINOGEN ACTIVATOR RELEASE, SMOKING AND ATHEROSCLEROSIS

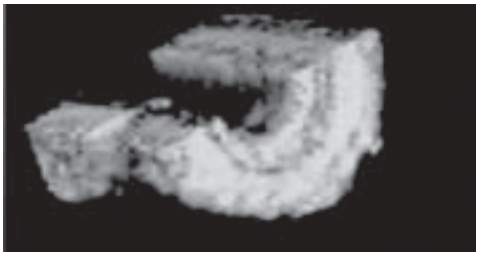
To address this issue, we assessed the blood flow and t-PA responses to selective intracoronary infusions of substance P and sodium nitroprusside in patients with coronary artery disease using intracoronary ultrasound and Doppler combined with coronary sinus sampling.[28] Coronary angiography is an inaccurate quantitative method of assessing atherosclerotic plaque load because it can only assess the arterial lumen and is unable to take account of 'Glagovian' arterial remodelling where the artery expands to accommodate the atheroma. We, therefore, determined the plaque volume of the left anterior descending coronary artery using computerised three-dimensional reconstruction of the intravascular ultrasound images (Figure 2).

The acute local fibrinolytic capacity of the coronary circulation was determined in 25 patients with coronary artery disease, and we were able to demonstrate a direct relationship between acute local coronary release of t-PA and both the coronary atheromatous plaque burden and smoking habit (Figure 3).[28] These important findings suggest that both atherosclerosis and smoking habit adversely influence the local fibrinolytic balance in the coronary circulation and provide a direct link between endothelial dysfunction and atherothrombosis.

This is the first clinical study to attempt to assess directly the acute release of t-PA in the coronary circulation and has found it to be sensitive to the presence of atheroma: a rapid decline in release of active t-PA associated with an increasing plaque burden. The mechanisms underlying this relationship remain to be established but are likely to involve chronic endothelial cell injury and, possibly, an impairment of the L-arginine:nitric oxide pathway.[26] In addition, this association may reflect a chronic stimulation and up-regulation of basal t-PA release caused by arterial denudation and atheroma.[29] The subsequent



Cross-section of coronary artery showing intimal plaque (arrow).



Three-dimensional computerised reconstruction.

**FIGURE 2**
**Intravascular ultrasound measurement of coronary artery plaque volume.**

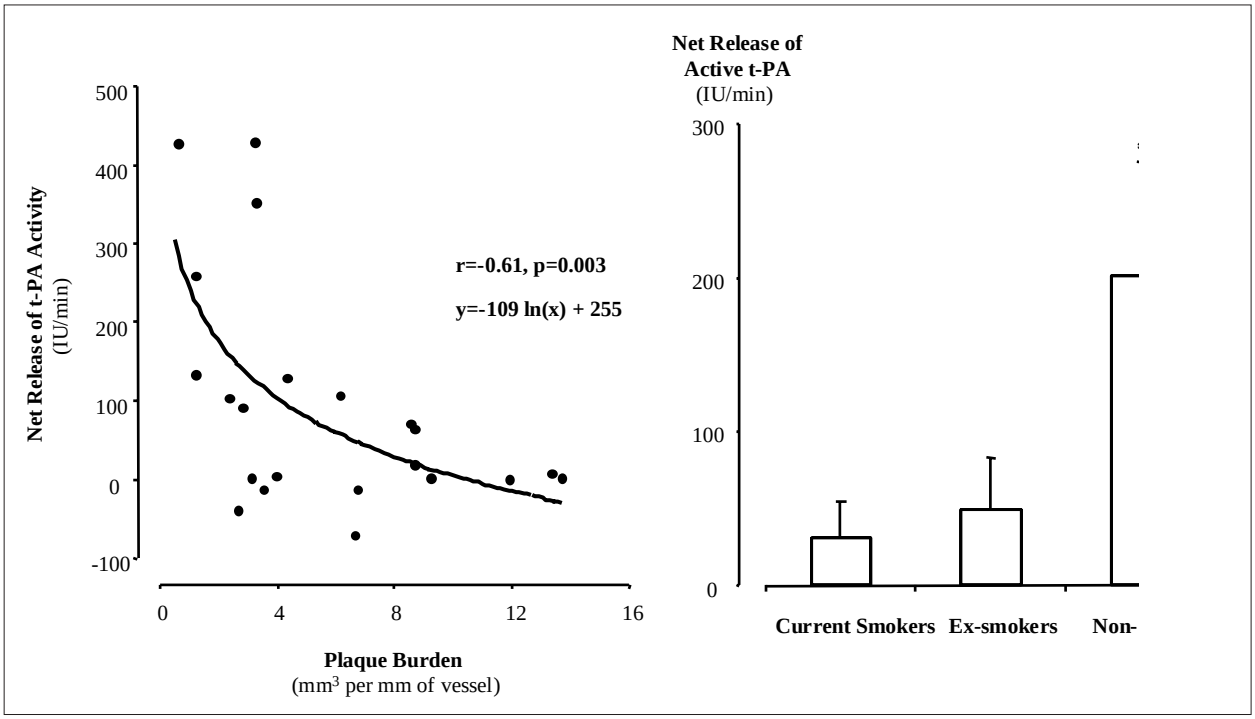



**FIGURE 3**
**Impaired endogenous coronary t-PA release associated with atherosclerosis and smoking habit.[28]**

depletion of endothelial cell t-PA stores, the associated increases in PAI-1 concentrations and the overall reduction of the acute dynamic fibrinolytic response would potentially limit the acute capacity of the vasculature to lyse intra-luminal thrombus. This is consistent with the epidemiological observations of a positive correlation between plasma t-PA and PAI-1 antigen concentrations and future coronary events,[30, 31] as well as our findings of an inverse correlation of active t-PA release with basal coronary sinus t-PA and PAI-1 antigen concentrations.

Questions of cause and effect cannot be resolved by this present study. Indeed, our observations are consistent with a reduced fibrinolytic activity causing enhanced atherogenesis. Detailed post-mortem studies have shown that plaque growth is induced by episodic subclinical plaque disruption and thrombus formation.[32] The prolonged presence of residual thrombus over a disrupted or eroded plaque will provoke smooth muscle migration and the production of new connective tissue, leading to plaque expansion.[32, 33] This is consistent with the enhanced macrovascular fibrin deposition and atherogenesis seen in genetic murine models of t-PA and plasminogen deficiency.[4, 5] However, it is likely that both processes, impaired fibrinolysis and atherogenesis, cooperate and interact to damage vascular function and structure.

## CLINICAL IMPLICATIONS

This work provides a novel method of assessing endothelial function that is directly relevant to atherogenesis and its thrombotic consequences. The

findings of impaired fibrinolysis in cigarette smokers provide an intriguing explanation for the smokers' paradox. In addition, this detailed series of studies provides the first clinical evidence to support the hypothesis of Rosenberg and Aird[2] that vascular bed-specific defects in haemostasis exist, and that coronary thrombosis critically depends on the local fibrinolytic balance. Moreover, our observations are also consistent with a reduced fibrinolytic activity causing enhanced atheroma formation, and are supported by the findings of genetic murine models where atherogenesis is accelerated by t-PA and plasminogen deficiency.

The identification of the mechanisms involved, and of therapeutic strategies to enhance the acute release of endogenous tissue plasminogen activator, will be of major clinical relevance. Current systemic administration of fibrinolytic agents is associated with significant bleeding complications, including cerebral and gastrointestinal haemorrhage. In addition to the potential prevention of thrombotic cardiovascular events, selective enhancement of local endogenous fibrinolysis is likely to be more therapeutically effective as well as avoiding the serious haemorrhagic complications associated with the use of systemic fibrinolytic agents. Moreover, enhanced endogenous fibrinolysis is likely to have a beneficial preventative and anti-atherogenic effect that warrants further detailed investigation.

**ACKNOWLEDGEMENTS**

The work undertaken by Dr David Newby, and upon which this article is based, was supported by the British Heart Foundation.

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