Uremic Serum and Ubiquitylation of Tissue Factor

Running title: Mackman; Tissue factor and stent thrombosis

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Stent thrombosis is a major clinical problem associated with a high rate of mortality.\(^1\)\(^2\) The development of drug-eluting stents (DES) has significantly reduced restenosis compared with bare metal stents (BMS) due to the antiproliferative agents inhibiting vascular smooth muscle (VSMC) proliferation. However, the rate of stent thrombosis associated with DES continues to be a major concern with rates between 0.3 and 1.1% within 3 years.\(^1\) Thrombosis is likely in part due to the fact that the antiproliferative drugs also inhibit re-endothelization of the vessel. Furthermore, patients with chronic renal failure have worse outcomes after stenting and have as much as an 8 fold increase in 1-year cardiac mortality following coronary intervention.\(^3\)

Tissue factor (TF) is a transmembrane protein that triggers blood coagulation.\(^4\) It has been proposed that TF plays a role in stent thrombosis, although there is no direct evidence for this assumption in patients. Platelet binding to the damaged vessel with or without exposure of TF is equally likely to trigger thrombosis. Chitalia et al\(^5\) have made the interesting observation that uremic serum increases TF protein stability in VSMCs. They suggest that this may explain the increase in cardiac mortality in chronic renal failure patients receiving stents.

In healthy vessels, TF is separated from its ligand factor VII/VIIa in the blood by the endothelium and this prevents inadvertent activation of blood coagulation. In the normal vessel wall high levels of TF mRNA and protein are expressed by adventitial fibroblasts where it provides hemostatic protection.\(^6\) TF mRNA and protein were below the detection limit in VSMCs but one study found a low level of TF activity in normal rat aortic media.\(^7\) In animal models, endothelial denudation without medial injury is associated with platelet deposition without fibrin, whereas fibrin is observed with more severe injury that damage the media.\(^8\)\(^9\) These results suggest that exposure of adventitial TF is required for activation of blood coagulation and fibrin deposition. Surprisingly, deletion of the TF gene in VSMC in mice
reduced ferric chloride-induced thrombosis.\textsuperscript{10} This suggests that VSMCs express low levels of functional TF that can induce thrombosis in some models.

Pathological expression of TF can trigger thrombosis in a variety of diseases. For instance, TF is expressed by macrophages and VSMCs within atherosclerotic plaques and likely contributes to thrombosis after plaque rupture.\textsuperscript{11} Bacterial lipopolysaccharide induces TF gene expression in monocytes and this activates the coagulation cascade.\textsuperscript{12,13} Lipopolysaccharide induces TF gene expression in monocytes by activating various transcription factors, including NF-κB.\textsuperscript{14} Furthermore, the increase in TF protein expression in monocytes and endothelial cells was in part due to an increase in TF mRNA stability.\textsuperscript{15,16} TF expression in VSMCs is induced by variety of growth factors and cytokines, including platelet-derived growth factor.\textsuperscript{17} The antiproliferative drugs rapamycin and paclitaxel in DES also induce TF expression in VSMCs.\textsuperscript{1} However, in stimulated VSMCs only 20\% of the TF is expressed on the cell surface.\textsuperscript{18} Interestingly, TF expression in vascular cells, such as monocytes, endothelial cells and VSMCs, is generally transient due to shut down of gene expression and rapid turnover of TF mRNA and protein. Similarly, TF expression is transiently expressed in a balloon-injured rat aortic media.\textsuperscript{7} Finally, cell surface TF activity is regulated by various mechanisms including exposure to phosphatidylserine.\textsuperscript{19} Therefore, there are at least 4 levels of regulation of cell surface TF expression and activity (\textbf{Figure 1}).

Chitalia et al\textsuperscript{5} discovered that uremic serum increased levels of TF protein in VSMCs without increasing TF mRNA expression. This was a somewhat unexpected finding since a previous study found that uremic serum increased TF mRNA and protein expression in endothelial cells.\textsuperscript{20} Chitalia et al\textsuperscript{5} found that the half-life of TF protein was significantly prolonged in the presence of uremic serum. Recent studies have shown that ubiquitylation of
membrane proteins negatively regulates their cell surface expression by directing protein
degradation via the proteasome pathway.\textsuperscript{21} One class of receptors that are regulated by
ubiquitylation are the cytokine receptors. Indeed, TF is a member of this class of receptors.\textsuperscript{4}
Treatment of VSMC with a proteasome inhibitor revealed that TF was ubiquitylated. Importantly,
uremic serum reduced the level of TF ubiquitylation, which likely explains the increased half-life
of the protein. Interestingly, the uremic solutes indole-3-acetic acid, indoxyl sulfate and uric acid
also increased TF protein expression in VSMCs. However, only indole-3-acetic acid, indoxyl
sulfate inhibited TF ubiquitylation, indicating that uric acid increased TF protein expression via
an undefined mechanism. In addition, it is not know what receptors and intracellular pathways
mediate indole-3-acetic acid and indoxyl sulfate inhibition of TF ubiquitylation.

The choice of VSMCs is relevant for studies in stent thrombosis. However, it should be
noted that cultured human VSMCs express high basal TF expression \textit{compared} with VSMCs in
vivo. It would be interesting to examine TF expression in the wall of stented vessels from
patients with or without chronic renal failure. In addition, other cell types may express TF within
the injured vessel wall, such as macrophages, and these could also contribute to stent thrombosis.
The human VSMCs were studied in a flow-loop model that mimics coronary flow. Cells were
exposed to control or uremic serum for 24 hours. No time-course experiments were presented
and the time points used to examine TF mRNA levels were not reported. One complication with
the study is that serum is a strong inducer of TF expression in VSMCs. It is likely that the initial
exposure of the cells to serum would transiently increase TF expression. Indeed, levels of TF
protein and activity were higher in cells exposed to 10\% serum compared to cells exposed to 5\%
serum. Surprisingly, 10\% uremic serum induced higher levels of TF protein (3.3 fold) than TF
activity (2 fold), whereas there was a similar increase (2 fold) of TF protein and activity in cells
exposed to 5% uremic serum. This suggests that TF protein is increasing without a corresponding increase in TF activity. Unfortunately, the method employed to measure TF activity was not reported. Further, the TF activity of VSMCs exposed to 5 or 10% of control or uremic serum was not compared in the clot assay.

The triggers of stent thrombosis remain a mystery. What is particularly perplexing is that thrombosis can occur at a considerable time after deployment of the stent. Further studies are needed with animal models and patients to identify the sources of the prothrombotic material. Obviously, stents continue to be improved and increasing re-endothelization will reduce rates of thrombosis. At present, patients receiving stents are treated with platelet inhibitors to prevent thrombosis but additional antithrombotic strategies may be beneficial in the future.

**Conflict of Interest Disclosures:** None.

**References:**


**Figure Legend:**

**Figure 1.** Cell surface TF expression and activity is regulated at multiple levels. TF can be regulated at the level of the gene, mRNA, protein or activity. * represents active TF.
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