Venous Thromboembolism – The Intersection of Thrombosis and Inflammation

Pathogenesis of Venous Thromboembolism

Venous thromboembolism (VTE) is a significant healthcare problem in the US. It has been estimated that there are >900,000 cases of deep venous thrombosis (DVT) and pulmonary embolism (PE) yearly, with approximately 300,000 deaths. Over 150 years, thoughts on the pathogenesis of VTE centred around Virchow’s triad of stasis, changes in the vessel wall and thrombogenic changes in the blood. However, in the early 1970s, through the pioneering theories of Gwendylen Stewart, a relationship between thrombosis and inflammation, and vice versa, was suggested.

Today, there is ample evidence of such a relationship. Inflammation affects coagulation in a number of ways, including an increase in tissue factor (TF), membrane phospholipids, platelet reactivity and fibrinogen. Additionally, inflammation downregulates thrombomodulin, the receptor for proteins C and Z, vascular heparins and activated protein C half-life, and increases levels of plasminogen activator-1 (PAI-1), thus decreasing thrombolysis. In this article, we will review the role that inflammation has with regard to thrombosis, as assessed with various animal models, emphasising the role of P-selectin and pro-coagulant microparticles (MPs).

Inflammation and Thrombosis

After VTE is initiated, an acute to chronic inflammatory response occurs in the vein wall and thrombus. This inflammation leads to amplification of the thrombus along with thrombus organisation and thrombus recanalisation. This inflammatory response occurs at the expense of vein wall and vein valvular damage. Initially, there is an increase in neutrophils in the vein, followed by monocytes/macrophages (see Figure 1). Cytokines, chemokines and inflammatory factors such as tumour necrosis factor-alpha (TNF-α) facilitate inflammation. The ultimate response of the vein wall depends on pro-inflammatory and anti-inflammatory mediators. We have found that selectins (P- and E-selectin) are integrally involved in this process. Selectins are the first upregulated glycoproteins on activated endothelial cells and platelets. They are expressed by activated platelets from alpha granules and endothelial cells from Weible-Palade bodies.

Activating signals such as thrombin and histamine lead to the rapid redistribution of P-selectin to the surface of cells in a ligand-receptive form. The receptor for P-selectin is P-selectin glycoprotein ligand-1 (PSGL-1), a homodimeric mucin present on the majority of leukocytes. The binding of P-selectin to its receptor initiates signalling through various pathways and mediates leukocyte–endothelial cell, leukocyte–platelet and leukocyte–leukocyte interactions. Even platelets roll and tether onto stimulated endothelial cells from this interaction.

We used both rat and mouse models of inferior vena cava (IVC) thrombosis in studies of the basic mechanisms of thrombogenesis and thrombus resolution. The cell-adhesion molecule P-selectin has been found upregulated in the vein wall as early as six hours after thrombus induction, while E-selectin was upregulated at day six after thrombosis, with appropriate increases in gene expression preceding the protein elevations. The natural anti-inflammatory cytokine interleukin-10 was upregulated in a fashion that countered the inflammatory response noted.

In order to further define the significance that the selectins have on the inflammatory and thrombotic response, genetically modified knockout (KO) mice have been studied in which either P- or E-selectin or both P- and E-selectin were deleted. In these studies, deletion of E-selectin and combined P-selectin/E-selectin deletion was associated with decreased thrombosis, while the vein wall inflammatory response was most inhibited in the combined P-selectin/E-selectin and P-selectin KO groups.

P-selectin, Microparticles and Thrombogenesis

We have studied the influence of elevated levels of soluble P-selectin on MPs using a mouse termed the delta cytoplasmic-tail (ΔCT) mouse. This mouse demonstrated four-fold elevations in circulating soluble P-selectin.
A significant 50% increase in IVC thrombus mass was noted in this mouse at days two and six after thrombosis, and this increase was associated with the increase of pro-coagulant MPs in the circulation, most prominently from leukocyte origin (see Figure 2). Animals deficient in P- and E-selectin had decreased thrombosis (−16%) and MPs. MPs are fragments of phospholipid cell membranes that promote coagulation and modulate a number of inflammatory cell–vessel wall interactions. MPs have on their surface phosphatidylserine, TF and the P-selectin receptor PSGL-1. MPs have been shown to be pro-coagulant in a number of in vitro models, and their presence has been associated with normal physiology along with peripheral vascular occlusive disease, unstable angina, angina before and after percutaneous transluminal coronary angioplasty (PTCA), ischaemic stroke, severe trauma and sepsis. Platelet-derived MPs are involved in thrombosis in heparin-induced thrombocytopenia. Less is known regarding leukocyte-derived MPs, although they are associated with endothelial cell activation and cytokine gene induction. Additionally, MPs derived from endothelial cells cause monocyte TF antigen to be released, thus increasing TF expression.

The thrombogenic potential of MPs is dependent on TF expression and their anionic, pro-thrombotic surface capable of assembling pro-thrombinase and tenase. We have been able to show that MPs associated with animals with thrombosis produce more TF when measured by flow cytometry than MPs obtained from control non-thrombosed animals. We have also shown a strong correlation (ratio (r) = 0.99) between the number of MPs and the amount of TF expression on the MP surface (see Table 1).

In previous studies, we have confirmed the importance of P-selectin and its receptor PSGL-1 in VTE using a primate model of stasis-induced IVC thrombosis produced by a temporary six-hour balloon occlusion. In this model, we have found that an antibody to P-selectin or a receptor antagonist (termed rPSGL-Ig) inhibits inflammation and thrombosis when given prophylactically. A significant dose–response relationship between rPSGL-Ig and thrombosis (p<0.01) and rPSGL-Ig and spontaneous recanalisation (p<0.05) has been demonstrated. No systemic anticoagulation, bleeding-time prolongation, thrombocytopenia or wound-healing complications were found in those animals treated with rPSGL-Ig. Direct selectin inhibition also effectively treats VTE in a primate model of iliofemoral DVT formation. In the first study, we compared rPSGL-Ig, dalteparin, low-molecular-weight-heparin (LMWH) (Fragmin®) and saline control using magnetic resonance venography (MRV) to quantify per cent recanalisation. Dalteparin and rPSGL-Ig were equally effective in promoting recanalisation, but the former caused significant increases in coagulation function while the latter caused no changes in coagulation function. Both treatments also preserved iliac vein valvular function.

We have also documented the ability of an oral P-selectin inhibitor given prophylactically. A significant dose–response relationship between rPSGL-Ig and thrombosis (p<0.01) and rPSGL-Ig and spontaneous recanalisation (p<0.05) has been demonstrated. No systemic anticoagulation, bleeding-time prolongation, thrombocytopenia or wound-healing complications were found in those animals treated with rPSGL-Ig. Direct selectin inhibition also effectively treats VTE in a primate model of iliofemoral DVT formation. In the first study, we compared rPSGL-Ig, dalteparin, low-molecular-weight-heparin (LMWH) (Fragmin®) and saline control using magnetic resonance venography (MRV) to quantify per cent recanalisation. Dalteparin and rPSGL-Ig were equally effective in promoting recanalisation, but the former caused significant increases in coagulation function while the latter caused no changes in coagulation function. Both treatments also preserved iliac vein valvular function.

We have also documented the ability of an oral P-selectin inhibitor given prophylactically. A significant dose–response relationship between rPSGL-Ig and thrombosis (p<0.01) and rPSGL-Ig and spontaneous recanalisation (p<0.05) has been demonstrated. No systemic anticoagulation, bleeding-time prolongation, thrombocytopenia or wound-healing complications were found in those animals treated with rPSGL-Ig. Direct selectin inhibition also effectively treats VTE in a primate model of iliofemoral DVT formation. In the first study, we compared rPSGL-Ig, dalteparin, low-molecular-weight-heparin (LMWH) (Fragmin®) and saline control using magnetic resonance venography (MRV) to quantify per cent recanalisation. Dalteparin and rPSGL-Ig were equally effective in promoting recanalisation, but the former caused significant increases in coagulation function while the latter caused no changes in coagulation function. Both treatments also preserved iliac vein valvular function.

We have also documented the ability of an oral P-selectin inhibitor given prophylactically. A significant dose–response relationship between rPSGL-Ig and thrombosis (p<0.01) and rPSGL-Ig and spontaneous recanalisation (p<0.05) has been demonstrated. No systemic anticoagulation, bleeding-time prolongation, thrombocytopenia or wound-healing complications were found in those animals treated with rPSGL-Ig. Direct selectin inhibition also effectively treats VTE in a primate model of iliofemoral DVT formation. In the first study, we compared rPSGL-Ig, dalteparin, low-molecular-weight-heparin (LMWH) (Fragmin®) and saline control using magnetic resonance venography (MRV) to quantify per cent recanalisation. Dalteparin and rPSGL-Ig were equally effective in promoting recanalisation, but the former caused significant increases in coagulation function while the latter caused no changes in coagulation function. Both treatments also preserved iliac vein valvular function.

We have also documented the ability of an oral P-selectin inhibitor given prophylactically. A significant dose–response relationship between rPSGL-Ig and thrombosis (p<0.01) and rPSGL-Ig and spontaneous recanalisation (p<0.05) has been demonstrated. No systemic anticoagulation, bleeding-time prolongation, thrombocytopenia or wound-healing complications were found in those animals treated with rPSGL-Ig. Direct selectin inhibition also effectively treats VTE in a primate model of iliofemoral DVT formation. In the first study, we compared rPSGL-Ig, dalteparin, low-molecular-weight-heparin (LMWH) (Fragmin®) and saline control using magnetic resonance venography (MRV) to quantify per cent recanalisation. Dalteparin and rPSGL-Ig were equally effective in promoting recanalisation, but the former caused significant increases in coagulation function while the latter caused no changes in coagulation function. Both treatments also preserved iliac vein valvular function.
Thrombosis and Haemostasis


Conclusion

Thrombosis and inflammation are inter-related. Selectins are important in thrombogenesis, and it is possible to target inflammation to limit thrombus amplification. Further development of this approach should result in the development of agent(s) that can be used for prophylaxis and/or treatment of DVT.

Acknowledgement

Supported in part by R01 HL070766 (TWW).

**Figure 3: Vein Opening**

The same inflammatory response documented in the prime is also seen in patients. Levels of P-selectin have been found to be elevated in DVT patients, and are decreased with heparin treatment. Additionally, in a series of 544 consecutive patients with recurrent idiopathic DVT/PE using soluble P-selectin at >75th percentile, patients had a 1.7-fold increased risk of recurrence a minimum of three months after the initial thrombotic event. The measured levels of P-selectin were well past the time at which they should have been influenced by the acute thrombosis, suggesting that elevated levels of P-selectin may function as a pro-coagulant factor in the blood. Finally, we have attempted to use biomarker levels of soluble P-selectin, pro-coagulant MPs, and a D-dimer in an algorithm to diagnose DVT. We have increased sensitivity and specificity to 73 and 81%, respectively, with this combination.

We hope to come up with other combinations to increase the sensitivity/specificity of biomarkers to make the diagnosis of DVT match the effectiveness of diagnostic duplex imaging.

period better than enoxaparin sodium, again with no change in coagulation function (see Figure 3). Vein wall inflammation was decreased as measured by MR imaging of gadolinium extravasation into the vein wall.

These data suggest that with initial thrombosis, selectin upregulation leads to MP formation; the MPs are then recruited into the area of the vessel free of thrombus. The pro-coagulant nature of these MPs was demonstrated by their ability to normalise bleeding in factor VIII-deficient mice. Additionally, there is growing evidence that blood TF associated with leukocytes, or circulating in a soluble form, is also involved in venous thrombogenesis. Such blood-borne capability may relate to MPs. However, we have also demonstrated that although this blood-borne TF exists, vessel wall TF is also driving the formation of venous thrombosis in our rodent model of stasis-induced DVT.

The same inflammatory response documented in the prime is also seen in patients. Levels of P-selectin have been found to be elevated in DVT patients, and are decreased with heparin treatment. Additionally, in a series of 544 consecutive patients with recurrent idiopathic DVT/PE using soluble P-selectin at >75th percentile, patients had a 1.7-fold increased risk of recurrence a minimum of three months after the initial thrombotic event. The measured levels of P-selectin were well past the time at which they should have been influenced by the acute thrombosis, suggesting that elevated levels of P-selectin may function as a pro-coagulant factor in the blood. Finally, we have attempted to use biomarker levels of soluble P-selectin, pro-coagulant MPs, and a D-dimer in an algorithm to diagnose DVT. We have increased sensitivity and specificity to 73 and 81%, respectively, with this combination.

We hope to come up with other combinations to increase the sensitivity/specificity of biomarkers to make the diagnosis of DVT match the effectiveness of diagnostic duplex imaging.

Conclusion

Thrombosis and inflammation are inter-related. Selectins are important in thrombogenesis, and it is possible to target inflammation to limit thrombus amplification.

**Figure 3: Vein Opening**

<table>
<thead>
<tr>
<th></th>
<th>CTR</th>
<th>PSI-697</th>
<th>Enoxaparin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 2</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Day 6</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

* PSi-9 versus CTR day 2, p<0.01.
* Enoxaparin versus CTR day 2, p<0.01.

Combined proximal and distal segments of iliac veins from each primate were evaluated. Animals receiving enoxaparin sodium did not develop thrombosis at the time of balloon occlusion and demonstrated significant vein opening at day 2 (82±7 versus 0±0%; p<0.01) and day 6 (78±8 versus 0±0%; p<0.01). Animals receiving enoxaparin sodium did not form a clot. Notably, animals receiving PSI-697 compared with CTR demonstrated a significant increase in vein re-opening by magnetic resonance venography (MRV) at day 2 (78±10 versus 0±0%; p<0.01) and day 6 (78±8 versus 0±0%; p<0.01).

Animals receiving enoxaparin sodium did not form a clot. Notably, animals receiving PSI-697 compared with CTR demonstrated a significant increase in vein re-opening by magnetic resonance venography (MRV) at day 2 (78±10 versus 0±0%; p<0.01) and day 6 (78±8 versus 0±0%; p<0.01). Animals receiving enoxaparin sodium did not develop thrombosis at the time of balloon occlusion and demonstrated significant vein opening at day 2 (82±7 versus 0±0%; p<0.01) and day 6 (78±8 versus 0±0%; p<0.01) compared with controls.

**Conclusion**

Thrombosis and inflammation are inter-related. Selectins are important in thrombogenesis, and it is possible to target inflammation to limit thrombus amplification. Further development of this approach should result in the development of agent(s) that can be used for prophylaxis and/or treatment of DVT.

**Acknowledgement**

Supported in part by R01 HL070766 (TWW).