
DISCUSSION PAPER

Thrombotic Thrombocytopenic Purpura - An Unfinished Story

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Three quarters of a century since the description by Eli Moschcowitz of the clinical phenomenon now recognised as TTP¹, its pathogenesis is still debated. The diagnostic pentad composed of neurological abnormalities, fever, renal dysfunction, thrombocytopenia and Microangiopathic Haemolytic Anaemia (MAHA) is increasingly replaced by the more pragmatic dyad of thrombocytopenia and MAHA for therapeutic intervention. This is largely due to the availability of plasma exchange or plasma infusion to treat this once largely fatal disorder².

Haemolytic Uraemic Syndrome (HUS) initially described as a fatal renal cortical necrosis in children shares with TTP the thrombocytopenia, MAHA and renal impairment³. The distinction between the two entities has long been debated. In the one prospective study where the two entities were distinguished based on the degree of renal impairment, no difference was observed in the prevalence of either the haematological or neurological impairment between the two groups⁴; furthermore the survival figures were similar. The impression that HUS unlike TTP responds poorly to plasma exchange may have arisen from the observation that unlike the haematological and neurological phenomena the recovery of renal function is slow and often incomplete⁵.

Both familial and sporadic forms of TTP/HUS are recognised. Estimates of disease recurrence vary with the underlying trigger of the initial event. Lack of uniformity in the definition of the interval between cessation of plasma exchange and disease recurrence makes comparative estimates erroneous. A recurrence rate of 20% for idiopathic TTP was observed in a study by Vesely et al, 1996. TTP is often idiopathic but may be triggered by an infection-typically a diarrhoeal illness from a Shiga family-toxin producing strain of *E. coli* or drugs - Mitomycin C, Cyclosporin, Pentostatin, Gemcitabine, Quinine and more recently Ticlopidine and

Clopidogrel. TTP/HUS associated with pregnancy, HIV infection and allogeneic bone marrow transplantation have been described.

Theories that relate to the pathogenesis of TTP are based on a series of observations. The fibrin poor, von Willebrand Factor (vWF) rich platelet aggregates in the arteriolar and capillary beds characterise the lesion in TTP¹. The vascular beds are devoid of inflammatory change and endothelial cell necrosis is uncommon in the initial phase but becomes prominent as the disease progresses⁷. Exposure of cultured EC to TTP plasma results in cell apoptosis as evidenced by an increase in FAS (CD 95)⁸. This in-vitro data is supported by the evidence of Microvascular Endothelial Cell apoptosis in spleens removed from patients with TTP⁹. It is suspected that the primary insult in TTP results in endothelial cell apoptosis with release of a factor or factors that help propagate platelet aggregation. It is proposed that ultra large vWF multimers constitute one such factor¹⁰.

vWF is a multimeric plasma protein synthesised by endothelial cells and megakaryocytes¹¹ which helps platelets adhere to the subendothelium of damaged blood vessels, initiating the formation of a haemostatic plug. Multimer formation takes place in the endoplasmic reticulum. Dimers are first assembled from pairs of 250kDa polypeptide subunits via disulfide bridges between cysteine residues located in the carboxy terminal regions. Subsequently, multimers are formed by interdimeric disulfide linking of amino terminal domains¹². Only the large multimeric forms of vWF are haemostatically active¹³. The unusually large vWF multimers secreted by endothelial cells have been shown to be more effective than the largest plasma forms in inducing platelet aggregation under conditions of high fluid shear¹⁴. This consequence of multimer size relates to the affinity of vWF for its ligands. Multimeric vWF binds with a ~100 fold greater affinity to both collagen and platelets than monomeric vWF. Modulation of vWF multimer size is therefore critical to the control of its

haemostatic activity. Moake proposed in 1982 that proteolytic cleavage of vWF subunits or reductions of the disulfide bonds linking vWF dimers were possible mechanisms by which the size of vWF multimers is regulated.

Normal plasma has circulating fragments of vWF subunits corresponding to proteolytic cleavage between Tyr842-Met843⁽¹⁵⁾. A number of well studied proteases digest vWF in vitro (e.g. plasmin, leukocyte elastase and platelet calpain) but do not result in fragments corresponding to this particular site of cleavage. In 1996 Furlan and Tsai^{16,17} independently reported a novel metalloproteinase that cleaves vWF at this site. Its biological relevance was further strengthened by demonstrating a deficiency of this enzyme in 6 patients in 3 families with relapsing TTP and the presence of a neutralising IgG inhibitor to this metalloproteinase in 20 of 24 patients with sporadic TTP¹⁸. Normal protease activity was demonstrated in patients with HUS leading to an assertion by the authors that the two disorders are distinct and the presence or absence of the protease could help make the distinction. The criteria used in this study to assign patients into TTP or HUS groups are unclear.

Native vWF is resistant to degradation by this metalloproteinase unless first denatured by Guanidine HCl¹⁷. It has been suggested but not proven that high shear stress in vivo can result in a similar conformational change in vWF that allows its subsequent proteolytic cleavage¹⁷. Furthermore the vWF cleaving metalloproteinase has minimal activity unless treated with high concentrations of Ca²⁺ or Ba²⁺ ions¹⁸. Such unphysiological conditions are incongruous with an enzyme associated with a function as critical as the regulation of vWF multimer size.

There is compelling evidence to suggest that vWF multimer size is controlled by a vWF reductase secreted by endothelial cells¹⁹. Incubation of normal plasma with the conditioned medium of cultured macro and microvascular vascular endothelial cells for 24 hrs results in a decrease in the average multimer size of vWF; specifically the very large multimers were lost. A similar phe-

nomenon was observed with the plasma of a TTP patient. Human umbilical vein endothelial cells when stimulated by thrombin release endogenous vWF into its cultured medium. Analysis of the cultured medium at set intervals demonstrated that the average multimer size of secreted vWF was reduced with time. The substance in the cultured medium capable of reducing the average multimer size was inactivated by heat, implying that the factor was a protein. Importantly its activity was not inhibited by a series of proteinase inhibitors including phosphoramidon, a metalloproteinase inhibitor, but was neutralised by disulfide reductase inhibitors. Despite clear evidence of reduction in vWF multimer size there was no increase in the proteolytic fragments that would have resulted from cleavage at Tyr842-Met843. The reduction of multimer size was paralleled by an increase in free thiols corresponding to the uncoupling of disulfide bridges in vWF. This activity was replicated by two known disulfide reductases - thioredoxin and protein disulfide isomerase.

Thrombospondin 1 has recently been confirmed, in both in-vitro studies and in mice as the putative vWF reductase (Xie et al unpublished). TSP1, a major component of platelet alpha granules has hitherto been known for mediating cell-cell and cell-matrix interactions. It is proposed that TSP1 is involved in the normal control of vWF multimer size; perturbation of the secretion or function of TSP1 or polymorphism in the TSP1 gene may contribute to disorders characterised by altered vWF multimer size such as TTP and Type II vWD.

Proteolytic cleavage and the disulfide reduction of vWF are two separate mechanisms, by which vWF multimers are depolymerised. It is possible that the two mechanisms act in concert to modulate vWF multimer size.

TTP is a rare disease and now largely treatable if recognised early. The importance of the association between TTP and vWF multimer size extends beyond this particular disorder as it promises to shed light on the mechanism by which the balance between thrombosis and haemorrhage is maintained by the regulation of vWF multimer size.

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