REGIONAL CENTRE FOR BIOTECHNOLOGY
Seminar series

# A new mechanism of hemolysis - induced thrombosis and vascular occlusion in Sickle Cell Disease and Thalassemia-β; and development of anti-thrombotic therapeutics


## Prasenjit Guchhait, Ph.D
**Assistant Professor**
**Baylor College of Medicine**
**Houston, Texas - 77030, USA.**


**Monday, March 14, 2011**
**9:00 am**
**Seminar Room**

As the part of my long-standing interest in thrombosis and cardiovascular research (Barnerdo et al 2004; Lasser et al 2006; Guchhait et al 2007 and Guchhait et al 2008, Zhou et al 2011), I have recently pursued an interesting work to understand the complex pathogenesis of the intravascular clot formation and blood vessel occlusion in sickle cell disease (SCD) and thalassemia-β (Thal-β; Zhou et al 2009, Zhou et al 2010, Zhou and Guchhait 2011). Both hemolytic disorders are caused by a gene mutation in the β-globin gene of hemoglobin (Hb) and affect millions of people worldwide including a large population in India.

Though clinical manifestations of the diseases are quite heterogeneous, many of them (including thrombosis, thromboembolism, vasoocclusive crises, myocardial infarction, acute chest syndrome, and ischemic stroke) commonly occur in both diseases due to vascular occlusion mediated via blood cell adhesion to vascular endothelium. Investigating the cause of unusual cell adhesion to vessel wall, studies including our own, suggest that a plasma multimeric glycoprotein, von Willebrand factor (VWF) is crucially involved in the development of the above clinical crisis events in both diseases. Normally VWF serves the hemostatic functions including the blocking of bleeding etc., however under pathophysiological disease conditions in SCD and Thal-β, the hyperactive VWF multimers play the crucial role in the pathogenesis of the above crisis conditions by promoting unusual cell adhesion to blood vessel wall.

Our recent observations suggest that the hyperactive VWF multimers are accumulated in plasma of the patients of both diseases due to the inhibition of VWF cleavage by a plasma metalloprotease ADAMTS13. The excessive amount of extracellular hemoglobin (ECHb) in plasma of the patients of both diseases potentially inhibits the VWF cleavage by ADAMTS13. ECHb binding to VWF protects the multimers from cleavage by ADAMTS13 (Zhou et al 2009). Consistent with the notion, a subpopulation of Hb-bound VWF (HbVWF) multimers was isolated

from patients' plasma, is uncleavable by metalloprotease in vitro and is hyperadhesive to platelets and the subendothelial matrix protein collagen, indicating that the HbVWF multimers might play a crucial role in unusual platelet adhesion and clot formation in these patients (Zhou et al 2010, Zhou and Guchhait 2011). Furthermore, we observed a significant correlation between the plasma HbVWF and the crisis events in a small pool of SCD patients. The high plasma concentration of the hyperactive HbVWF multimers was further elevated during the vasoocclusive pain crisis in SCD patients (unpublished).

We further focused on translation of above molecular findings on diseases to therapeutic practice. We have designed a recombinant polypeptide VWFA2 (25 kDa; designed from the ECHb binding site on VWF) that potentially inhibits ECHb-VWF interaction in vitro; and have tested in vivo in transgenic mice with SCD. We observed that intravenous administration of VWFA2 polypeptide (1 mg/1 kg BW) significantly inhibited VWF accumulation on vascular endothelium in SCD mice, and has dramatically improved the mice survival (unpublished).

In summary, our observations show that intravascular-hemolysis promotes intravascular clot formation and vascular occlusion in patients with SCD/Thal-β by a mechanism where excessive plasma ECHb blocks the cleavage of the VWF by metalloprotease ADAMTS13, and promotes accumulation of the ultralarge and hyperadhesive Hb-bound VWF (HbVWF) multimers that culminates cell adhesion to vessel wall. The plasma HbVWF multimers level parallels with the vasoocclusive pain events and thrombotic complications in SCD patients. The recombinant polypeptide, VWFA2, which inhibits ECHb-VWF interaction in vitro, significantly reduces the VWF accumulation and activity in vivo in mice with SCD.

Thus, the above observations provide new insights into the several key areas in the pathogenesis of vascular occlusion, strokes, infarctions and thrombosis in patients with the hemolytic disorders specifically SCD and Thal-β. Study also suggests that this is probably a common mechanism for other related disorders too. The study also suggests that such focus on the relevance of ECHb-VWF-ADAMTS13 axes may lead to the development of targeted therapies for the above debilitating complications in these hemolytic disorders.