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DIFFERENTIAL ENDOTHELIAL VCAM1 EXPRESSION AND IMPLICATIONS FOR SICKLE CELL ANEMIA VASCULOPATHY

Topic: 26. Sickle cell disease

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Background: Vascular disease is systemic in sickle cell anemia (SCA), with profound effects in organs like the brain, where stroke is the most severe end of the cerebral vasculopathy spectrum. Endothelial dysfunction is an important pathobiological mechanism in SCA systemic vasculopathy, with upregulation of adhesion molecules (e.g., VCAM-1), lower nitric oxide bioavailability, and increased oxidative stress. In previous association studies, we found positive associations between the presence of three specific *VCAM1* gene promoter haplotypes and i) high blood flow velocities in the median cerebral artery, and ii) a chronic hemolysis biochemical marker.

Aims: The aims of our work were: a) to investigate the functional role of those *VCAM1* promoter haplotypes in endothelial cell response following endothelial activation through TNF-α stimulation; b) to assess the modulation role of proinflammatory and/or pro-oxidative stimuli on endothelial *VCAM1* expression; and, finally, to evaluate how hydroxyurea (HU) treatment would affect that expression.

Methods: After molecular cloning of three *VCAM1* promoter haplotype constructs, using pGL4 promoterless vectors, haplotype sequence was confirmed, by Sanger sequencing, prior to transfection. Transfection experiments for each construct were performed, with or without TNF-α stimulation, using EAhy926, and HBEC as macrovascular and microvascular endothelial cell models, respectively. Differences in promoter activity were assessed by luciferase reporter assay. RNA was extracted from non-transfected EAhy926 and HBEC cell cultures stimulated or not with TNF-a and/or hemin, and with or without HU treatment. RT-qPCR was performed to analyze *VCAM1* expression. *HMOX1* and *NOS3* were also analyzed for comparison purposes.

Results: Our results showed that two *VCAM1* promoter haplotypes, previously associated with pediatric cerebral vasculopathy and hemolysis in SCA, increased promoter activity in transfected and TNF-α-stimulated EA.hy926 and HBEC cells, consistent with a higher *VCAM1* expression in macro and microvascular settings. In non-transfected cells, we also observed TNF-a-induced *VCAM1* overexpression as well as heme-induced overexpression of *HMOX1* in both cell models. Heme did not affect *VCAM1* nor *NOS3* expression and the latter was also not affected by TNF-a stimulus. Hydroxyurea treatment lowered TNF-a-induced *VCAM1* and *NOS3* expression but did not affect heme-induced *HMOX1* expression.

Summary/Conclusion: These data further indicate that *VCAM1* haplotypes we previously associated with pediatric cerebral vasculopathy and hemolysis in SCA, induce higher *VCAM1* expression potentially affecting both cerebral and systemic vasculopathy risk. The differential endothelial expression of *VCAM1*, *NOS3*, and *HMOX1* after proinflammatory and/or pro-oxidative stimuli also reinforces their genetic modulation role in SCA systemic vasculopathy.

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