BLOOD FLOW CHARACTERIZATION IN SICKLE CELL DISEASE.

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ABSTRACT

Sickle cell patients and transgenic sickle mice have vascular inflammation. Vascular inflammation secondary to ischemia/reperfusion is hypothesized to play an essential role in altering blood flow and inducing vaso-occlusion. Dorsal skin fold chambers (DSFCs) which allow repeat visualizations of the subcutaneous vasculature were implanted in normal control mice (n=13) and transgenic mice (n=17) expressing human α and β/ $\beta^{S\text{-}Antilles}$ globins. Repeat measurements of venule diameters, blood flow velocities and leukocyte rolling were made on the same veins(n=36 control and n=72 sickle veins)using intravital microscopy(IVM). After baseline measurements, the mice were exposed to hypoxia (7% O2/93% N2) for 1 hour followed by 1 hour of re-oxygenation in room air. A third visualization was made after administration of sodium nitroprusside(SNP), a vasodilator. The wall shear rates were significantly higher in the sickle animals in the smaller veins (<40μm) than the normals and significantly lower in larger veins (>40 μm) than the normals. Wall shear rates did not vary significantly after 1 hour of hypoxia and 1 hour of re-oxygenation. Leukocyte (WBC) rolling was significantly higher at baseline in the sickle mice compared to the normals. A higher leukocyte rolling was also observed after 3 hours of hypoxia followed by 2 hours of re-oxygenation. In conclusion, these results demonstrate the potential of the DSFCs to allow repeat visualizations for observing blood flow changes in transgenic sickle mice. Future work will focus on the connection between inflammation and vaso-occlusion after hypoxia/re-oxygenation in this model.

INTRODUCTION

Sickle cell disease is characterized by recurring acute vaso-occlusive episodes and chronic damage to multiple organs. The substitution of valine for glutamate at the sixth position of the beta chain of hemoglobin (Hb) has been the focus of understanding of the pathophysiology of sickle cell disease. This point mutation results in the polymerization of HbS and sickling of red blood cells (RBCs) in vivo under deoxygenated conditions. Over the past two decades, the role of the endothelium and its interactions with the sickle RBCs has led to a revised paradigm for the understanding of vaso-occlusive phenomena in sickle cell disease [1].

Inflammation may play an essential role in vaso-occlusion in sickle cell disease. Sickle cell patients have high white counts and elevated levels of serum C-reactive protein, cytokines and adhesion molecules. We hypothesize that reactive oxygen species derived from sickle RBCs, with possible amplification by WBCs, and on going ischemia-reperfusion may produce vascular inflammation. This results in endothelial activation and the binding of RBCs and WBCs to the endothelial surface causing transient vaso-occlusion, in turn producing more ischemia-reperfusion injury in an ongoing vicious cycle.

Previous work on studying the blood flow dynamics in transgenic mice were performed on models such as mesentery and cremaster [2,3]. These models do not lend themselves to chronic studies. We have incorporated the DSFC model to observe blood flow changes in both normal and transgenic sickle mice. These chambers allow for repeat visualizations of the same vessels over time and also analysis of blood flow in them after different interventions such as hypoxia/re-oxygenation and anti-inflammatory drugs. In this study, we will be carrying out measurements of inflammation and blood flow parameters such as red blood cell velocity, venular diameter, stasis and WBC rolling flux in transgenic sickle and normal mice.

MATERIALS AND METHODS

Mice. Normal C57BL/6 mice were obtained from Jackson laboratories. Transgenic sickle mice [4] were produced by crossing a transgenic mouse line expressing high levels of human α and β^S chains with a line expressing human α and $\beta^{S\text{-}Antilles}$. These mice are homozygous for deletion of the mouse β-major globin.

Measurement of Serum amyloid P-component(SAP). Blood was collected from the tail vein before DSFC implantation and on days 3,4 and 7 after implantation. Serum was used to measure SAP by enzyme immunoassay [5].

DSFC implantation. The experimental model used in this study was originally developed for the rat dorsal skin flap [6] and modified

for mice. Animals were on antibiotic amoxicillin at least three days before chamber placement. The DSFCs were implanted as previously outlined in [7]. The animals were given 4 days recovery period.

IVM and analysis. Female C57BL/6 mice (n=13) and female transgenic sickle mice (n=17) weighing approximately 20g (10-12 weeks old) were used. The mice were anesthetized intraperitoneally with a ketamine-xylazine anesthetic mixture. IVM is performed as described elsewhere in [2]. Mean red cell velocity measurements are made as in [8], venular diameters as in [9] and WBC rolling as in [2].

Experimental protocol. Four days after dorsal skin fold chamber implantations, the mice(both normal and sickle) were visualized separately and the venular diameter, mean red cell velocity and leukocyte rolling was measured. The mice were then transferred to a special chamber and were subjected to 1 hour of hypoxia(7% O_2/93% N_2) followed by 1 hour of re-oxygenation in room air. A second visualization was made and the same parameters were re-measured on the same veins. Sodium nitroprusside was topically administered after this and the same parameters were measured on the same veins. The mice were then euthanized using a CO_2 chamber.

RESULTS

1. Serum amyloid P-component (SAP) is an acute phase response protein with 60-70% sequence homology to human CRP. SAP increases in normal mice 3 and 4 days after implantation of the dorsal skin fold chamber, but returns to baseline 7 days after surgery. In sickle mice SAP is elevated 8.5- to 12.1- fold ($p<0.001$) at baseline compared to normal mice.

2. Blood flow parameters obtained from the normal mice with implanted DSFCs were compared with other model systems previously used in literature [10]. The blood flow dynamics in these animals were seen to follow the same trend as shown below, as the venular diameter increased so did the mean RBC velocity.

3. Blood flow parameters in the veins in sickle mice were more chaotic than in the normal mice as seen from Fig.1.

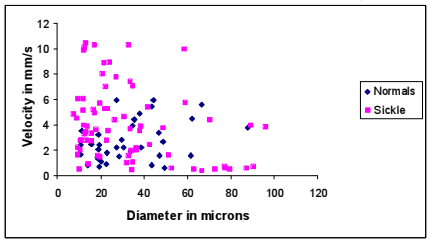

Figure 1. RBC velocity (mm/s) vs diameter (μm) in normal and sickle mice

4. Wall shear rates were calculated based on the formula $8V_{mean}/D$ [2]. Based on our data above, we divided over veins into two groups, small veins <40μm and large veins >40μm. Wall shear rates were significantly higher in the sickle mice in the smaller veins and significantly lower in larger veins when compared to the normal mice as shown in the plot below. Wall shear rates increased insignificantly after 1 hour of hypoxia and 1 hour of re-oxygenation and decreased insignificantly after SNP administration in both normal and sickle mice and in both classes of veins.

5. WBC rolling was significantly higher in baseline in sickle animals when compared to normals.(Fig 2) Also WBC rolling increased after 3 hours of hypoxia and 2 hours of re-oxygenation in sickle mice from baseline, though after 1hour of hypoxia and 1 hour of re-oxygenation no increase of WBC rolling was seen.

6. 9% of the veins after 1 hour of hypoxia and 1 hour of re-oxygenation were static in the sickle mice while none were static in the normal mice (Fig 3). No stasis was observed at baseline.

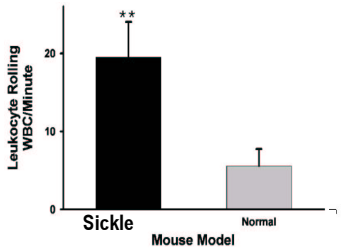

Figure 2. WBC rolling flux in sickle and normal mice at baseline.

Figure 3. Percentage stasis in sickle and normals after 1hr Hypoxia/1hr re-oxygenation.

DISCUSSION

The DSFC model allows visualization of the microcirculation over time unlike other models such as mesentery or cremaster. In conjunction with IVM, blood flow characterization and changes can be tracked on individual vessels. The main disadvantage of DSFCs are that they are themselves a source of inflammation as seen from SAP data in the normal mice (Result #1). The flow parameters obtained from normal mice were seen to obey the Murray's law where the flow rate varies as to d^3, where d is the mean diameter of the vessel. The sickle data was more chaotic and did not appear to follow Murray's law.

Longer hypoxia(3hr) and re-oxygenation(2hr) period increases WBC rolling in sickle mice (Result #5). This shows that a longer hypoxia/re-oxygenation was required to trigger more vascular inflammation in these mice. Nevertheless, stasis could be observed even after 1 hour of hypoxia and 1 hour of re-oxygenation. (Result #6)

The facts that even at baseline a higher rolling was observed in the sickle mice and a small amount of stasis was observed in these animals suggest that both the WBCs and the RBCs may play a critical role in the pathophysiology of vaso-occlusion in sickle cell disease [11]. The presence of a highly variable blood flow condition in these animals further suggest that these mice may be in a permanently "inflammed" state as previously hypothesized in [12].

Further studies on a more severe murine model [13] of sickle cell disease are planned to corroborate our hypothesis and our findings and the potential benefits of using an anti-inflammatory drug therapy for this disease.

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