MEDICAL SCHOOL
UNIVERSITY OF MICHIGAN

Capstone for Impact Submission | GY2020

Project Title: The Effects of Prolonged Anticoagulation with Low Molecular Weight Heparin on Fibrotic Vein Wall Remodeling in a Murine Model of Venous Thrombosis

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Branch: Procedure-based Care

Path of Excellence: Scientific Discovery

If this project can be continued by another UMMS student, please include your contact information or any other details you would like to share here:

Summary: Up to 50% of patients who undergo anticoagulation therapy for an un-provoked acute vein thrombosis develop post thrombotic syndrome (PTS), a fibrotic complication. There is a current debate on whether prolong anticoagulation can reduce PTS. Low molecular weight heparin (LMWH) has been shown to have anti-inflammatory properties, and it is thought that inflammation plays a major role in the development of PTS during thrombus resolution. The aim of this study was to determine if prolonged anticoagulation using low molecular weight heparin reduces fibrotic vein wall remodeling in a murine model of VT.

Methodology: Male, 10-12 weeks old, C57BL/6 mice underwent the Inferior Vena Cava (IVC) model and received standard anticoagulation (LMWH x 6 days + Saline + 8 days) or prolonged anticoagulation (LMWH X 14 days). The IVC model involved anesthetizing the mouse, performing a laparotomy, conducting a retroperitoneal dissection to gain access to the IVC, ligation of lateral and lumbar side branches, and consequent ligation of the IVC below the renal veins. LMWH dosing was 5.5 mg/kg twice daily, subcutaneously, post-surgery. Day 14 post-thrombosis blood was drawn, and the IVC/thrombus was weighed and harvested for further evaluation with qRT-PCR of fibrotic markers (Col1a, Col3a1, TGF-beta1, FSP-1, MMP-2 and MMP-9) or histology with Masson's Trichrome staining. ImageJ was used to analyze histologic images, and a script was generated to select only pixels positive for fibrosis. These pixels were then standardized to the sum of pixels of the vein walls, and results were reported as fibrotic area per vein wall cross-section.

Results:

Thrombus weight was significantly decreased for both LMWH x6 days (14mg ± 0.89mg) and LMWH x14 days (12mg ± 0. 52mg) when compared to LMWH x2 days (25mg ± 0.26mg) and saline groups (23mg ± 0.55mg). There was no difference in thrombus weight between the LMWH x6 days and LMWH x14 days groups. The mean vein wall collagen area to vein wall area ratios decreased as the anticoagulation treatment was prolonged (saline 0.631 ± 0.078; LMWH x2 days 0.555 ± 0.086; LMWH x6 days 0.357 ± 0.054; LMWH x14 days 0.313 ± 0.065). The collagen deposition of the LMWH x14 days treatment group was significantly decreased in comparison to both the saline and LMWH x2 days groups. There was no significant difference

in gene expression of MMP-2, MMP-9, Col1a1, and TGF-beta1 among all four groups. However, Col3a activity was significantly decreased for LMWH x14 days at 0.59 ± 0.21 when compared LMWH x 2 days and LMWH x 6 days (saline 5.16 ±0.72; LMWH x 2 days 8.64 ± 1.85, LMWH x6 days 9.24 ± 2.22). FSP-1 expression was also significantly decreased for LMWH x 14 days at 0.0767 ± 0.248 when compared to LMWH x 2 days (0.145 ± 0.010).

Conclusion: Extending anticoagulation decreased mean vein wall fibrosis. Thrombus weight, collagen deposition, Col3a1 and FSP-1 were significantly decreased with prolonged anticoagulation of LMWH x 14 days. Thus, extending anticoagulation decreases fibrotic vein wall remodeling in a mouse model of occlusive VT.

Reflection/Impact Statement:

This research project significantly challenged me to understand the basic science of thrombus resolution and fibrotic remodeling. In order to perform appropriate rT-PCR, a thorough understanding of acute phase reactants and markers of fibrosis was necessary. Patients could significantly benefit from this project if it was replicated in non-human primate models of venous thrombosis, as we demonstrated prolonged anticoagulation decreased overall vein wall fibrosis. This has implications that PTS would be less likely to develop or be less severe in patients who received prolonged anticoagulation with LMWH. Although my PI has recently taken a position at a different university, I think any students who are interested in vascular surgery could use this project as preliminary data in order to justifiy a retrospective study looking at length of anticoagulation and PTS in patients who suffered venous thrombosis.