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An *In Vivo* Murine Model of Low Magnitude Oscillatory Wall Shear Stress to Address the Molecular Mechanisms of Mechanotransduction

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Abstract

Objective—Current understanding of shear sensitive signaling pathways has primarily been studied *in vitro* largely due to a lack of adequate *in vivo* models. Our objective was to develop a simple and well characterized murine aortic coarctation model to acutely alter the hemodynamic environment *in vivo* and test the hypothesis that endothelial inflammatory protein expression is acutely upregulated *in vivo* in by low magnitude oscillatory WSS.

Methods and Results—Our model utilizes the shape memory response of nitinol clips to reproducibly induce an aortic coarctation and allow subsequent focal control over WSS in the aorta. We modeled the corresponding hemodynamic environment using computational fluid dynamics and showed that the coarctation produces low magnitude oscillatory WSS distal to the clip. To assess the biological significance of this model, we correlated WSS to inflammatory protein expression and fatty streak formation. VCAM-1 expression and fatty streak formation were both found to increase significantly in regions corresponding to acutely induced low magnitude oscillatory WSS.

Conclusions—We have developed a novel aortic coarctation model that will be a useful tool for analyzing the *in vivo* molecular mechanisms of mechanotransduction in various murine models.

Keywords

Wall Shear Stress; Coarctation; Mechanotransduction; Atherosclerosis; Murine Model

Atherosclerosis is an inflammatory disease of the vasculature that is predisposed to localization in regions uniquely characterized by disturbed blood flow and the resultant low

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magnitude oscillatory Wall Shear Stress (WSS) [1, 2]. Mechanosensitive pathways have therefore been implicated as important mediators in the pathogenesis of cardiovascular disease. Previous studies have identified likely mechanisms of mechanotransduction and downstream signaling pathways as summarized in numerous reviews [3]. However, experimental flow models have largely been limited to *in vitro* methods, which have highly simplified, non-physiologic, flow environments, lack complex cell-cell and cell-matrix interactions, and have variable conditions (cell line, time course, culture conditions, etc.) between studies. These limitations have led to uncertainties in the applicability of these mechanotransduction pathways to *in vivo* conditions.

Mouse models of disturbed flow are beginning to be used for the *in vivo* analysis of molecular mechanisms of mechanotransduction. These models fall into two categories: chronic or device-based. Chronic models utilize regions where the innate morphology produces chronically disturbed flow, including the aortic arch and the brachiocephalic branch [4]. The complex morphology in these regions makes for challenging analysis while the chronic flow environment allows for activation of compensatory mechanisms; thus, studies are limited to the analysis of the atherosusceptible endothelial phenotype or lesion development. Alternatively, device-based models can acutely disturb flow and isolate WSS induced signaling from chronic compensatory mechanisms [5, 6]. While these models have provided insights into the molecular mechanisms involved, the associated hemodynamic environments are very complex. Critical limitations include large alterations of pressure (or lack of characterization of pressure) resulting in an unintended mechanical stimulus, or no oscillations in flow resulting in a WSS profile that differs from many *in vivo* regions of pathogenesis. Due to these complexities, there is currently a need for an easily implemented and highly reproducible, acute, *in vivo* model of low magnitude oscillatory WSS in which molecular mechanisms of mechanotransduction can be analyzed. To address this need, we hypothesized that utilizing a nitinol clip, we could reliably produce a mouse coarctation model that would induce quantifiable acute changes in wall shear stress which would subsequently result in increased expression of flow-mediated inflammatory proteins.

Methods

Detailed methods related to animal care, nitinol clip manufacturing, surgical methodology, Computational Fluid Dynamics (CFD), and histology are described in the supplemental data section. Briefly, we used shape memory nitinol clips with an inner diameter smaller than the aortic diameter of a mouse. The aorta of anesthetized mice was exposed and a nitinol clip deformed to an open state (Figure 1 A) was inserted underneath the aorta. The body temperature of the mouse thermally activated the shape memory recovery of the clip thereby decreasing the aortic diameter and inducing an aortic coarctation. A CFD model was then created to determine the hemodynamic environment near the coarctation. To assess the biological significance, we stained for either VCAM-1 expression over an acute time course or fatty streak formation over a chronic time course.

Results

Coarctation Model

We found that nitinol clips could be used to effectively induce an aortic coarctation. See supplemental results sections for the characterization of the coarctation.

Characterization of the Hemodynamic Environment

WSS maps and cross sectional velocity vectors were generated using a CFD model for control, non-coarctation aortas and coarctation aortas (n=3) (Figure 1 & Supplemental

Figure III). These models showed unidirectional and relatively high magnitude WSS in the upstream, thoracic, region of control and coarcted mice. The spatial heterogeneity of the WSS was relatively minimal with some skewing towards the posterior side due to the curvature of the spine. Downstream from the site of the aortic coarctation the model showed low magnitude oscillatory WSS on the anterior side of the aorta, while the control animals showed unidirectional flow with high magnitude WSS. Low magnitude oscillatory WSS was observed in aortic coarctation CFD models from three different mice, demonstrating the reproducibility of this flow environment .

From these results we identified three regions of interest: the thoracic aorta, the low magnitude oscillatory WSS region in the abdominal aorta of mice from the coarctation group, and the comparable abdominal region near the celiac branch in control animals. We included the aortic arch as an additional region of interest based on previous studies showing chronic induction of low oscillatory WSS in this region [4]. This animal model can therefore compare regions of high magnitude unidirectional WSS with regions of both acutely and chronically disturbed flow producing low magnitude oscillatory WSS.

Biological Markers

We used quantum dot-based immunohistochemistry to quantify the expression of a representative inflammatory protein, VCAM-1, in our model (n=8-10). Control non-coarcted aortas and control aortas with a sham, non-constricting clip, showed low levels of VCAM-1 expression throughout the celiac region of the aorta (Figure 2). Aortas with a coarctation inducing nitinol clip showed a significant increase in VCAM-1 expression in the region of disturbed flow as identified earlier. This increase in VCAM-1 expression was comparable to VCAM-1 expression in regions of chronically disturbed flow and significantly higher than regions of steady laminar flow (Figure 2).

We used ApoE$^{-/-}$ mice on a normal chow for 2 months as a model of early lesion formation (n=5). Low levels of fatty streaks formed in the control animals and animals with a sham, non-constricting, clip while fatty streaks formation significantly increased in the disturbed flow region of the coarctation model (Figure 2).

Discussion

Our study presents a novel mouse model of acutely disturbed flow. We have thoroughly characterized the hemodynamic environment associated with our model. Using a combination of techniques, *in situ* microCT and *ex vivo* pressure inflation, we showed that we can effectively constrict the aortic diameter within the nitinol clip with minimal variability. The difference between the two measurements can be attributed to the thickness of the vessel wall which is included in the *ex vivo* inflation measurements. We further showed that this constriction creates a small degree of stenosis. This stenosis produced minimal pressure drop across the coarctation, as confirmed by blood pressure measurements. The CFD model showed that the coarctation produced low magnitude oscillatory WSS in the distal region. This coarctation model is therefore a model which can uniquely analyze the response to acute changes in WSS without a significant effect on blood pressure. We further show that the coarctation produces acute inflammatory protein expression as well as chronic fatty streak formation. Mice with a sham, non-constricting, clip showed that the biological response, both VCAM-1 expression and fatty streak formation, was a product of the altered hemodynamic environement and not the perivascular tissue dissection or an inflammatory response to the nitinol material. The acute shear response downstream from the coarctation can also be compared to the chronic shear response in the aortic arch, thereby allowing for analysis of compensatory mechanisms and the atherosusceptible endothelial phenotype.

We are currently limited in our in vivo imaging methodologies. Using MR we cannot obtain images at the clip due to magnetic susceptibility artifacts, while ultrasound is similarly not possible due to the presence of the clip. To address these limitations we utilized an *ex vivo* perfusion set up to measure the compliance of the aorta. The inflation experiment was used to show that the aortic diameter is constricted within the coarctation and that the clip does not deform under a luminal physiologic blood pressure. Additionally we performed a sensitivity analysis on the boundary conditions to show that within a physiologic blood pressure range, our parameters (outflow pressure and expansion factor) do not affect the observed flow reversal, though there is some change in the heterogenous distribution of WSS.

We conclude that this model provides a number of advantages over existing *in vivo* mouse models of disturbed flow including: simple implementation, high reproducibility, a well characterized low oscillatory WSS environment, negligible effect on pressure, and both an acute and a chronic disturbed flow environment. This novel model will be an important tool in which to assess the *in vivo* applicability of shear sensitive signaling pathways, already determined through *in vitro* experimentation. The availability of transgenic and knock out animals make mice an ideal animal to investigate these mechanosensitive molecular mechanisms. Further understanding of how these pathways act *in vivo* will be important in the development of new therapies to treat cardiovascular disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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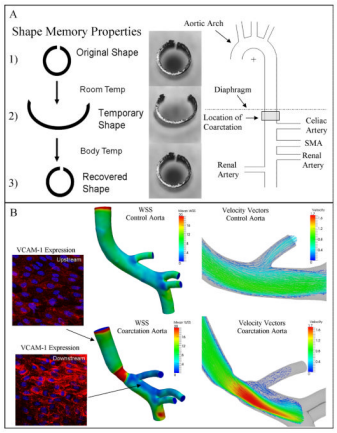

Figure 1.

Panel A shows the three stage shape memory response of a nitinol clip and a diagram of the aorta showing the location of coarctation (right). Panel B shows representative confocal images (left) of the endothelium stained with Qdots conjugated to anti-VCAM-1 antibodies (red). Representative mean WSS (Pascals) maps (center) and velocity (m/s) vector maps (right) are shown during the downstroke of systole. Maps were generated from CFD models of a control mouse aorta (upper images) and a mouse aortic coarctation (lower images).

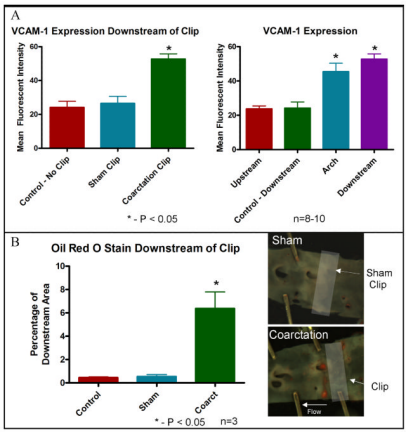

Figure 2.

Panel A shows the quantified fluorescent intensity of the quantum dots representative of VCAM-1 expression. The right plot shows VCAM-1 expression in various regions of the aorta including the chronically disturbed flow region in the lesser curvature of the aortic arch [4] and the acutely disturbed flow region downstream from the coarctation (n=8-10, $p<0.05$). Panel B shows fatty streak formation as measured by oil red o staining (n=5, $p<0.05$).