Proceedings of the ASME 2013 Summer Bioengineering Conference
SBC2013
June 26-29, 2013, Sunriver, Oregon, USA

SBC2013-14475

REDUCTION IN MECHANICAL WALL STRAIN PRECEDES INTIMAL HYPERPLASIA FORMATION IN A MURINE MODEL OF ARTERIAL OCCLUSIVE DISEASE

John T. Favreau[1], Chengwei Liu[2,3], Peng Yu[2], Christine Mauro[2], Ming Tao, C. Keith Ozaki[2], Glenn R. Gaudette[1]

[1]Department of Biomedical Engineering
Worcester Polytechnic Institute
Worcester, MA 01609
USA

[2]Division of Vascular and Endovascular Surgery
Brigham and Women's Hospital
Boston, MA 02115
USA

[3]Division of Vascular Surgery
The First Affiliated Hospital of Jiamusi University
Jiamusi, Heilongjiang, 154003
China

INTRODUCTION

Coronary artery disease and peripheral artery disease remain a significant source of mortality and vascular morbidity in the United States; both affecting over 14 million Americans.[1] Although a number of both open and endovascular procedures are available for treating occlusive lesions, post-procedure intimal hyperplasia (IH) and pathological wall adaptation in treated arteries cause further need for treatment. As on average 50% of patients receiving these treatments must receive further vascular intervention to prevent the continued expansion of IH into the vessel lumen, there is a need to improve our understanding of the underlying causes of IH formation.[2]

Recent research suggests that the mechanical environment applied to the arterial wall may play a critical role in the location and severity of IH and atherosclerosis. Studies have demonstrated that low wall shear stress,[3] high wall stress[4] and the combination of both factors[5] may play a role in the formation of IH in the vessel wall. Since extensive computational modeling is necessary to measure in vivo wall stress, in this study, the effects of cyclic mechanical wall strain on the resultant IH formation will be examined.

In order to model IH in vivo, it is first necessary to choose an in vivo model of IH formation. For this study, a previously developed distal focal stenosis model in the mouse was chosen.[6] This method was shown to consistently develop IH in the ~3mm segment of artery proximal to the focal stenosis. To measure mechanical strains non-invasively, a recently published approach, which utilizes high-frequency ultrasound, was used to determine strains at several time points.

In this study, we aimed to elucidate the relationship between acute changes in circumferential wall strain four days after stenosis creation with resulting changes in IH formation 28 days later.

MATERIALS AND METHODS

Murine Focal Stenosis Model

Eight-week-old C57BL/6J mice (n=10) were used for this study. Animals were imaged 1 day prior to operation, 4 days after operation and 27 days after operation. Animals were sacrificed at day 28.

Focal stenoses were created on the left common carotid artery as described previously.[6] Briefly, a 9-0 nylon suture was tied around both a 35 gauge blunt needle and the left common carotid artery in vivo (Figure 1, Black arrow). After removal of the needle, the ligature leaves an approximately 78% reduction in lumen diameter. In a previously published paper, this reduction was shown to cause the formation of clinically relevant IH in the ~3 mm segment proximal to the focal stenosis.[6]

Ultrasound Strain Measurement

Mechanical wall strains in the circumferential direction were measured in 3 locations (Figure 1, green lines) at each time point using a previously described method.[7] Briefly, mice were anesthetized using 1.25-1.5% isoflurane in oxygen and placed in a supine position. A 50/70 MHz linear array ultrasound transducer and Vevo2100 high frequency ultrasound machine with minimum spatial resolution of 75 microns, were used to acquire a 500 frame cine loop of the left common carotid artery in cross sections 2mm, 3mm and 4mm proximal to the carotid bifurcation (1mm, 2mm, and 3mm proximal to the focal stenosis, respectively). This procedure was repeated on the right (non-stenosed) side for all three regions. VevoStrain software was used to trace carotid artery circumferences and track changes in vessel circumference over time. All strain reported herein is mean dynamic strain in the circumferential direction from diastole to systole as measured over 5 consecutive heart beats.

Histological Analysis

On day 28 after focal stenosis creation, mice were euthanized and perfusion fixed. Both the right and left common carotid arteries were embedded in paraffin and serial cross-sections were cut for histological analysis. Using the carotid bifurcation and focal stenosis as landmarks, sections were selected approximately 2mm, 3mm, and 4mm from the carotid bifurcation. Sections were stained using Masson's trichrome and the lumen and internal elastic lamina (IEL) were identified and traced. Intimal thickness was computed by assuming that the measured circumferences were circular in vivo and given as the difference between the radius of the IEL and the radius of the lumen.

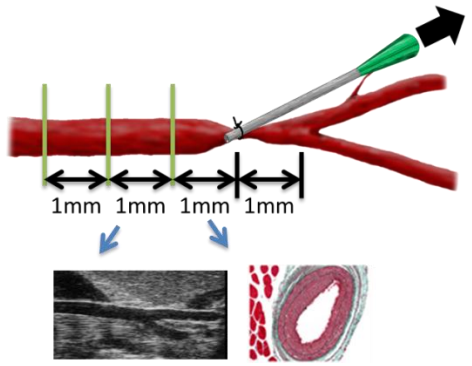

Figure 1: Location of Strain and histological measurements

Statistical Analysis

Comparisons between two groups were computed using a Student's t-test. Comparisons between 3 or more groups were computed using a one-way ANOVA with Tukey's post-hoc analysis. Results are reported as Mean ± SEM with significance at $p<0.05$.

RESULTS

Overall Trends in Strain

Four days after creation of the stenosis, mechanical strain decreased in all regions in the left common carotid (focal stenosis) from 0.26 ± 0.01 to 0.11 ± 0.02 (pre-op vs. day 4; $p<0.001$) with no change in the right common carotid (non-stenosed) compared to pre-op levels ($p=0.45$).

Relation Between Strain at Day 4 and Intimal Thickness

In addition to analysis of overall strain changes, acute regional differences in strain at day 4 were compared to the resultant IH formation at day 28. Due to poor image quality in some ultrasound images, 26 total segment strains at day 4 were compared to IH thickness at day 28. Based on a histogram of the strain levels, vessels were divided into groups with strain 0.1 or less and vessels with strain greater than 0.1. Of the 26 segments, all 13 regions with wall strain 0.1 or less at post-op day 4 had IH formation 28 days after focal stenosis creation. The average day 4 strain was 0.07 ± 0.01 with an intimal thickness of 31.5 ± 7.0 μm. In regions with mechanical strains >0.1 at day 4, 30% had formed IH at day 28 (Figure 2) mean strain 0.16 ± 0.01 and intimal thickness of 8.0 ± 4.0 μm.

Mean cross-sectional area at diastole of vessels with strain >0.1 remained constant from pre-op to day 4 scans (0.09 ± 0.01 vs. 0.09 ± 0.01 mm, $p=0.75$). However, in the strain <0.1 group diastolic area significantly increased from 0.10 ± 0.01 to 0.15 ± 0.01 mm ($p=0.03$). Mean cross sectional area at systole was seen to remain constant from pre-op to day 4 scans in both strain >0.1 group (0.15 ± 0.01 vs. 0.12 ± 0.02 mm, $p=0.09$) and strain <0.1 group (0.16 ± 0.01 vs. 0.17 ± 0.01 mm, $p=0.67$). Prior to focal stenosis creation, areas at both diastole and systole were the same in both groups ($p=0.37$ and 0.47, respectively)

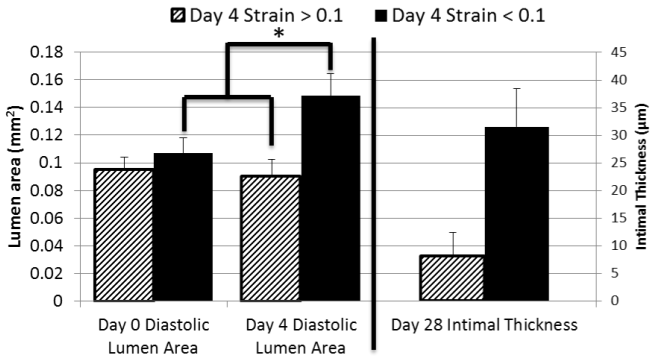

Figure 2: Differences in diastolic area and intimal thickness for animals with strain greater and less than 0.1 at day 4

DISCUSSION

The data presented herein have important implications for the field of vascular surgery. These results suggest that an acute reduction in dynamic mechanical strain may precede the formation of IH in this murine model. Since strains reported herein are measured only 4 days after focal stenosis creation, it is likely that little or no IH has formed at the time of strain recording. Additionally, acute changes in diastolic diameter imply wall adaptation soon after focal stenosis. These results suggest that it may be possible to noninvasively estimate the likelihood of IH formation using ultrasound imaging prior to IH formation.

ACKNOWLEDGEMENTS

This study was funded in part by the National Heart, Lung and Blood Institute grant T32HL007734, American Heart Association grants 12GRNT9510001 and 12PRE9020036 and by the Carl and Ruth Shapiro Family Foundation.

REFERENCES

[1] Go A. S., Mozaffarian D., Roger V. L., Benjamin E. J., Berry J. D., Borden W. B., Bravata D. M., Dai S., Ford E. S., Fox C. S., Franco S., Fullerton H. J., Gillespie C., Hailpern S. M., Heit J. a., Howard V. J., Huffman M. D., Kissela B. M., Kittner S. J., Lackland D. T., Lichtman J. H., Lisabeth L. D., Magid D., Marcus G. M., Marelli A., Matchar D. B., McGuire D. K., Mohler E. R., Moy C. S., Mussolino M. E., Nichol G., Paynter N. P., Schreiner P. J., Sorlie P. D., Stein J., Turan T. N., Virani S. S., Wong N. D., Woo D., and Turner M. B., 2013, "Heart Disease and Stroke Statistics--2013 Update A Report From the American Heart Association," Circulation, **127**, pp. e6–e245.

[2] Conte M. S., 2009, "Technical factors in lower-extremity vein bypass surgery: how can we improve outcomes?," Seminars in vascular surgery, **22**(4), pp. 227–33.

[3] Nanjo H., Sho E., Komatsu M., Sho M., Zarins C. K., and Masuda H., 2006, "Intermittent short-duration exposure to low wall shear stress induces intimal thickening in arteries exposed to chronic high shear stress.," Experimental and molecular pathology, **80**(1), pp. 38–45.

[4] Salzar R. S., Thubrikar M. J., and Eppink R. T., 1995, "Pressure-induced mechanical stress in the carotid artery bifurcation: a possible correlation to atherosclerosis.," Journal of biomechanics, **28**(11), pp. 1333–40.

[5] Zhao S. Z., Ariff B., Long Q., Hughes a D., Thom S. a, Stanton a V, and Xu X. Y., 2002, "Inter-individual variations in wall shear stress and mechanical stress distributions at the carotid artery bifurcation of healthy humans.," Journal of biomechanics, **35**(10), pp. 1367–77.

[6] Tao M., Mauro C. R., Yu P., Favreau J. T., Nguyen B., Gaudette G. R., and Ozaki C. K., 2012, "A Simplified Murine Intimal Hyperplasia Model Founded on a Focal Carotid Stenosis.," The American journal of pathology, **182**, pp. 277–287.

[7] Favreau J. T., Nguyen B. T., Gao I., Yu P., Tao M., Schneiderman J., Gaudette G. R., and Ozaki C. K., 2012, "Murine ultrasound imaging for circumferential strain analyses in the angiotensin II abdominal aortic aneurysm model.," Journal of vascular surgery, **56**(2), pp. 462–9.