Published in final edited form as:

J Vasc Interv Radiol. 2011 August ; 22(8): 1193–1197. doi:10.1016/j.jvir.2011.02.032.

Ultrasound-Guided Creation of a Small Animal Model of Aortic Injury

Xiaochun Meng^{1,2,*}, Feng Zhang^{1,*}, Karim Valji¹, Daohai Xie¹, Huidong Gu¹, Tiffany Blair¹, Xubin Li¹, Bensheng Qiu¹, Hong Shan², and Xiaoming Yang^{1,**}

¹Image-Guided Bio-Molecular Intervention Research and Section of Vascular & Interventional Radiology, Department of Radiology, University of Washington School of Medicine, Seattle, WA, USA 98109

²Department of Radiology, the 3rd Hospital of Sun Yat-sen University, Guangzhou, Guangdong, China 510630

Abstract

Purpose—To develop a small animal model of controlled aortic intimal injury with ultrasound imaging guidance.

Materials and Methods—Via carotid artery cut down, we advanced a custom-made micro-catheter/angled-metal-device system to damage the intima of the ascending aortas of 20 Sprague Dawley (SD) rats and 10 JCR atherosclerotic rats. This minimally invasive endovascular procedure was monitored by a clinical ultrasound imaging system. Injured aortas were harvested for histologic confirmations using a grading system: Grade I with intima injury, Grade II with injury to media, and Grade III with injury through the entire aortic wall. Neointimal reactions at the injury site were compared by calculating the ratio of intimal to medial thickness among different animal groups at various survival times (week 1, weeks 2–3 and weeks 4–7).

Results—Clear visualization of the architectures of the heart, great vessels and the exact location of the angled-metal-device by ultrasound imaging ensured consistent intimal damage of the aorta. Histopathology confirmed that most of the aortic injuries were classified as Grade I. There was no significant difference between the two rat groups. Analysis on pathophysiological reactions at the injury sites revealed increased thickening of neointimal hyperplasia as animal survival times extended from week 1 to weeks 4–7 after the aortic interventions.

Conclusions—This study demonstrates the feasibility of using clinical ultrasound imaging to precisely guide the creation of controlled aortic intimal injury in rats, which may become a useful tool to facilitate research involving the prevention and treatment of atherosclerotic cardiovascular disease.

Keywords

atherosclerotic cardiovascular disease; rat model; ultrasound; vascular injury

© 2011 The Society of Interventional Radiology. Published by Elsevier Inc. All rights reserved

**Correspondence to: Xiaoming Yang, MD, PhD Image-Guided Bio-Molecular Intervention Section Department of Radiology University of Washington School of Medicine Seattle, Washington, USA. xmyang@u.washington.edu.

*With equal contributions as co-first authors.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

INTRODUCTION

Laboratory animal model is one of the key components for basic research involving atherosclerotic cardiovascular disease — the leading cause of death in developed countries (1). Successful development of small animal models (such as mice and rats) with arterial injuries or atherosclerotic lesions offers great opportunities for investigating the pathogenesis, prevention, and treatment of atherosclerosis. However, to date, there is no ideal small animal model of arterial injury or atherosclerosis that simulates human lesions. Most existing small animal models of arterial disease are created by mechanical transmural injury (e.g., crushing with surgical forceps) (2) or endovascular mechanical denudation of normal vascular intima or the fibrotic caps of atherosclerotic plaques (3). In conjunction with the endovascular injury, a stimulus agent (such as p53 gene) can be locally injected into the atherosclerotic lesions to induce acute plaque cell death and thereby cause the site to become unstable and eventually rupture (4, 5). In general, creation of such models has required operative procedures with limited use of imaging guidance (6, 7). The aim of this study was to explore a method of using ultrasound imaging to guide creation of a rat model with controlled aortic injury.

MATERIALS AND METHODS

US-guided creation of aortic injury

We used two groups of rats: (i) 20 Sprague Dawley (SD) rats (300~470g, Harlan Laboratories, USA); and (ii) 10 JCR:LA-cp/cp atherosclerotic rats (5~7 weeks, Charles River Labs, USA) that were fed with an atherogenic diet for 11~12 months. All animal experiments were carried out in accordance with the National Institutes of Health guidelines and approved by the Institutional Animal Care and Use Committee.

Under general anesthesia, the right carotid artery was surgically exposed, and proximal and distal Sentinel loops (Sherwood Medical, St Louis, Montana) were applied for temporary vascular control. Transverse arteriotomy was performed in the exposed artery with Vannas style iris spring scissors (Roboz Surgical Instrument Co., Gaithersburg, Maryland). Through the arteriotomy, a 0.014-inch conventional guidewire (Terumo Co., Tokyo, Japan) was advanced into the ascending aorta. Then, a custom-made, 2F micro-catheter was advanced into the ascending aorta over the guidewire. Through the micro-catheter, the conventional guidewire was exchanged for a custom-made, 0.014-inch angled-metal-device, which had a 7-mm long and 120°-angled tip. Under ultrasound imaging (as described below), the micro-catheter/angled-metal-device system was advanced until the tips of the catheter and the angled-metal-device reached the aortic root. After withdrawing the micro-catheter back to the innominate artery, the intima of the aorta was damaged by withdrawing the angle-metal-device over a distance of approximately 5 mm. For each animal, the injury process was repeated 5–10 times by rotating the tip of the angled-metal-device in different directions (Figure 1). After creation of aortic wall injury, we removed the micro-catheter/angled-metal-device system, ligated the accessed carotid artery, and then closed the skin with 6-0 sutures. The animals were then returned to the cage and monitored daily through the experiments.

Ultrasound Imaging Guidance

The endovascular procedure for aortic wall injury was monitored using a clinical real-time ultrasound imaging system with an auto-adjustable 8–15 MHz linear-array transducer (SonoSite 180PLUS, Sonosite Inc, Bothell, Washington). We initially determined an optimal view, which best demonstrated the structures of interest such as the ascending aorta, the pulmonary trunk, and the cardiac ventricles. Then, under ultrasound guidance in this

optimal projection, injury of the ascending aortic wall was recorded as advancing and withdrawing the micro-catheter/angled-metal-device system.

Histology

To evaluate the histologic response of the injured aorta, the rats were euthanized at different phases, including (i) an acute sacrifice within 24 hours post-aortic injury for examining immediate effects of the model's creation (n=8); (ii) a sacrifice within one week for short-term effects (n=9); (iii) a sacrifice within 2–3 weeks for mid-term effects (n=7); and (iv) a sacrifice within 4–7 weeks for long-term effects (n=6). All sacrificed animals underwent an endovascular perfusion-fixation with 4% paraformaldehyde via an open-chest, left cardiac ventricle puncture approach. For the SD rats, the ascending aorta specimens were immediately kept in -80°C refrigerator. For the JCR rats, the ascending aortas were immediately stored in 4% paraformaldehyde with 0.1-M phosphate buffered saline (PBS) at 4°C . All of the harvested aortic specimens were embedded in an Optimal Cutting Temperature (OCT) compound (Sakura Finetek USA, Inc., Torrance, California), cryosectioned at 8- μm thickness, and then stained with (i) hematoxylin and eosin (H&E) staining; and/or (ii) Trichrome staining. Under microscopy, we then graded the histologic findings as (i) Grade I for aortic injury limited to the intima including the internal elastic lamina; (ii) Grade II for aortic injury extending to the media; and (iii) Grade III for injury through the entire aortic wall. We also examined neointimal hyperplasia (or repair) at the injured site (6, 7). Cross-sectional microscopy images of the aortas were captured with an Olympus DP70 CCD camera attached to an Olympus BX51 microscope (Olympus, Nagano, Japan). On the microscopic images of the thickest aortic walls, we measured the maximal thicknesses of the intima and the media using a Photoshop 7.0 software (Adobe Systems Incorporated, San José, USA). Subsequently, we calculated the average thickness of the intima and media, and then determined the average ratio that was thickness of intima / thickness of media (I/M ratio). This ratio enabled us to determine degrees of neointimal hyperplasia and thereby compare the pathophysiological reactions among different animal groups sacrificed at various times after the aortic injuries.

Data analysis

Three authors analyzed and recorded the aortic injury grades and neointimal reactions independently. Only the grade that met with consensus by two or more investigators was accepted. Statistical analysis was performed using the SPSS11.5 software (SPSS, Chicago, Illinois). The difference of the injury grade between SD rats and JCR rats was analyzed using two-independent samples test (Mann-Whitney test). The significant difference of I/M ratios among different animal groups sacrificed at various times was determined using ANOVA. A P value <0.05 was considered statistically significant.

RESULTS

US-guided creation of aortic injury

The procedure of ultrasound-guided aortic injury was successfully performed in all animals. During or within 24 hours after the operations, three of 20 SD rats died due to bleeding from the accessed carotid artery and one of 10 JCR rats died due to diffuse multi-organs infarction of unknown etiology.

In all cases, ultrasound imaging enabled us to clearly identify the right innominate artery, the ascending aorta and its root, the pulmonary trunk, and the cardiac ventricles (Figure 2). Under ultrasound imaging guidance, we could precisely position the tips of the micro-catheter and the angled-metal-device at the level of the aortic root, and then damage the

aortic intima under real-time imaging by withdrawing the angled-metal-device over a distance of approximately 5 mm.

Histology

Histologic grading showed that most of the aortic injuries were classified as Grade I. Aortic injuries of Grade II were found in two SD rats and one JCR rat. No Grade-III injury was found in either rat group. The thickened atherosclerotic aortic walls of JCR rats displayed accumulation of foamy macrophages with thrombosis deposits at the injured sites (Figure 3). Regarding the grading of the aortic injury, there was no statistically significant difference between the SD rat and JCR rat groups ($Z = 0.000$, $P > 0.05$).

Analysis of histologic changes at the injured sites showed the discontinuation of intimal layers with no neointimal reaction after the acute sacrifice. The neointimal hyperplastic reactions became more exuberant as the animal survival intervals increased. Histologically, the intima at the injured sites appeared to be re-endothelialized one week after the endovascular procedure, with several layers of infiltrative endothelial cells covering the injured intima. The I/M ratios were 0.21 ± 0.017 at week 1, 0.46 ± 0.044 at weeks 2–3, and 0.51 ± 0.024 at weeks 4–7. The I/M ratios at weeks 2–3 and weeks 4–7 was significantly higher than that at week 1 ($p < 0.05$) (Figures 4&5).

Discussion

Due to the very small caliber and thin wall of the major artery of a rat and mouse, the creation of an atherosclerotic plaque survival model based on intimal or subintimal injury without transmural rupture is extremely difficult. The results of the current study demonstrate that endovascular procedures for the creation of aortic wall injuries in such small animals can be successfully controlled under real-time ultrasound imaging guidance. We could not only visualize the architectures of the great vessels and the entire heart, but also the exact location of the micro-catheter/angled-metal-device system, which ensured consistency in damaging the aortic intima. In addition, the results of this study show that most of the aortic injuries in both animal groups were confined to the intimal layer (Grade I).

Reparative neointimal hyperplasia began at about week 1 after the injury and increased to 4–7 weeks after the initial insult. The reaction beyond this time period was not evaluated in this study. The histologic characteristics in this model is similar to that found with rat carotid arterial injuries induced by balloon angioplasty, shown as neointimal hyperplasia at the injured site (6, 7). These results indicate that the ultrasound-guided endovascular injury technique developed in this study may provide a useful approach for the controlled creation of aortic injuries in small animals.

Rats are the smallest widely available laboratory animals on which we can (i) employ various pediatric endovascular devices (e.g., micro-guidewires, micro-catheters); and (ii) monitor interventions with clinical ultrasound imaging. The ultrasound-guided aortic injury approach needs to be further validated in producing controlled “rupture” of atherosclerotic lesions that spontaneously develop in some species, such as JCR:LA-cp/cp rats (8, 9). Successful creation of such rat models may enable basic science research to investigate the mechanism of stabilization of vulnerable plaques and repair of ruptured plaques through application of novel modalities, including imaging-interactive regenerative medicine and gene therapy (10, 11). Further investigations are warranted to develop techniques which allow access to the rat carotid artery via a percutaneous rather than operative approach.

In conclusion, this study initially demonstrates the potential of using ultrasound imaging to precisely guide controlled creation of rat models of aortic injury. This method may become a useful technique for investigation of the prevention, diagnosis, and treatment of atherosclerotic cardiovascular disease.

Acknowledgments

This study was supported in part by NIH R01 HL078672 funding. Dr. Xiaochun Meng was supported by State Scholarship Fund of China Scholarship Council and the 3rd Hospital of Sun Yat-sen University.

References

1. American Heart Association. heart and stroke statistical update. American Heart Association; Dallas, TX: 2006.
2. Reddick RL, Zhang SH, Maeda N. Aortic atherosclerotic plaque injury in apolipoprotein E deficient mice. *Atherosclerosis*. 1998; 140:297–305. [PubMed: 9862272]
3. Rekhter M, Simari R, Work C, et al. Gene transfer into normal and atherosclerotic human blood vessels. *Circ Res*. 1998; 82:1243–1252. [PubMed: 9648720]
4. von-der-Thusen J, van-Vlijmen B, Hoeben R, et al. Induction of atherosclerotic plaque rupture in apolipoprotein E^{-/-} mice after adenovirus-mediated transfer of p53. *Circulation*. 2002; 105:2064–2070. [PubMed: 11980686]
5. Zhang L, Liu Y, Lu X, et al. Intraplaque Injection of Ad5-CMV.p53 Aggravates Local Inflammation and Leads to Plaque Instability in Rabbits. *J Cell Mol Med*. 2009; 13:2713–2723. [PubMed: 19602038]
6. Aizawa Y, Kawabe J, Hasebe N, et al. Pioglitazone enhances cytokine-induced apoptosis in vascular smooth muscle cells and reduces intimal hyperplasia. *Circulation*. 2001; 104:455–460. [PubMed: 11468209]
7. Razuvaev A, Lund K, Roy J, et al. Noninvasive real-time imaging of intima thickness after rat carotid artery balloon injury using ultrasound biomicroscopy. *Atherosclerosis*. 2008; 199:310–316. [PubMed: 18190918]
8. Brindley D, Russell J. Animal models of insulin resistance and cardiovascular disease: some therapeutic approaches using JCR:LA-cp rat. *Diabetes Obes Metab*. 2002; 4:1–10. [PubMed: 11890162]
9. O'Brien S, Kelly S, Dolphin P, et al. Chelation therapy in the JCR:LA-cp rat: experimental assessment of a putative antiatherosclerotic treatment. *Clin Invest Med*. 2000; 23:124–131. [PubMed: 10852662]
10. Qiu B, Yang X. Molecular MRI of hematopoietic stem-progenitor cells homing to atherosclerosis: A new avenue for in vivo monitoring of stem cell-mediated vascular gene therapy. *Nature CPCM*. 2008; 5:396–404.
11. Yang, X.; Lardo, A. MR imaging-guided cardiovascular interventions. In: Lardo, A.; Fayad, Z.; Chronos, N.; Fuster, V., editors. *Cardiovascular Magnetic Resonance: Established and Emerging Applications*. Martin Dunitz Taylor & Francis Group; London: 2003. p. 413-436.

Fig. 1. Schematic diagrams of ultrasound-guided endovascular injury of the rat's ascending aorta. (A–D) Via the right carotid artery cut down, an angled-metal-device (arrow on D) is positioned via a micro-catheter. Then, the intima of the aortic wall is damaged by withdrawing the angled-metal-device with its tip pointed in different directions (E–H).

Fig. 2.

Ultrasound-guided endovascular injury of the ascending aorta on a rat. (A) In an oblique ultrasound projection, the great vessels and the entire heart can be clearly seen. aAo=ascending aorta, RV=right ventricle, LV=left ventricle. (B) The location of the micro-catheter/angled-metal-device system and subsequent withdrawal of the device can be visualized and monitored with ultrasound imaging.

Fig. 3.

Representative histologic examinations from ultrasound-guided aortic injuries of rats.

(A&B) Microscopic images demonstrate a Grade I injury with a damaged intima (arrow on A) and a Grade II injury with the damage extending to the media (M)(arrow on B). (C)

Microscopic image shows a Grade II injury (arrow) with thrombus (T) deposit at the injured site of the aortic wall in a JCR rat. H&E staining, 20 $\times$.

Fig. 4. Representative histologic comparison of pathophysiological reactions at the injury sites of SD rats, showing increased neointimal hyperplasia (NH) and medial (M) ratios as the animal survival times increase from (A) week 1; (B) weeks 2–3; to (C) weeks 4–7 after the aortic interventions.

Fig. 5. Summary of the average intimal/media (I/M) ratios of various animal groups, demonstrating less intimal reaction in week 1 and increased intimal reactions in weeks 2–3 and weeks 4–7 (* $p < 0.05$). H&E staining, 20 $\times$.