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Authors

Stubbendorff, Mandy
Hua, Xiaoqin
Deuse, Tobias
et al.

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Video Article

Inducing Myointimal Hyperplasia Versus Atherosclerosis in Mice: An Introduction of Two Valid Models

Mandy Stubbendorff*[1,2], Xiaoqin Hua*[1,2], Tobias Deuse[1,2,3], Ziad Ali[4,5], Hermann Reichenspurner[2,3], Lars Maegdefessel[6], Robert C. Robbins[7], Sonja Schrepfer[1,2,3,4]

[1]Transplant and Stem Cell Immunobiology Lab, Cardiovascular Research Center, University Hospital Hamburg

[2]Cardiovascular Research Center (CVRC) and DZHK University Hamburg

[3]Department of Cardiovascular Surgery, University Heart Center Hamburg

[4]Center for Interventional Vascular Therapy, Division of Cardiology, Columbia University

[5]Cardiovascular Research Foundation, New York

[6]Karolinska Institute, Stockholm

[7]Department of Cardiothoracic Surgery, Stanford University School of Medicine, Falk Cardiovascular Research Center

*These authors contributed equally

Correspondence to: Sonja Schrepfer at schrepfer@stanford.edu

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Abstract

Various *in vivo* laboratory rodent models for the induction of artery stenosis have been established to mimic diseases that include arterial plaque formation and stenosis, as observed for example in ischemic heart disease. Two highly reproducible mouse models – both resulting in artery stenosis but each underlying a different pathway of development – are introduced here. The models represent the two most common causes of artery stenosis; namely one mouse model for each myointimal hyperplasia, and atherosclerosis are shown. To induce myointimal hyperplasia, a balloon catheter injury of the abdominal aorta is performed. For the development of atherosclerotic plaque, the ApoE -/- mouse model in combination with western fatty diet is used. Different model-adapted options for the measurement and evaluation of the results are named and described in this manuscript. The introduction and comparison of these two models provides information for scientists to choose the appropriate artery stenosis model in accordance to the scientific question asked.

Video Link

The video component of this article can be found at http://www.jove.com/video/51459/

Introduction

In industrialized nations ischemic heart disease remains a leading cause of mortality[1]. The causes for coronary artery stenosis are manifold and include myointimal hyperplasia as well as atherosclerosis, with revascularization remaining the most common treatment strategy[2]. Research models that clearly distinguish between the mechanistic pathways of intimal hyperplasia and atherosclerosis are essential to investigate the pathobiological and pathophysiological processes in arterial plaque development. In this video two different mouse models used to study either the development of myointimal hyperplasia or atherosclerosis are introduced.

Myointimal hyperplasia is postulated to form via the “response-to-injury” paradigm originally described for atherosclerosis[3]. The mechanical disruption of the endothelial layer leads to an intense remodeling enhanced by paracrine effects. There are several different animal models commonly used to study myointimal hyperplasia. Some groups use metal devices in the ascending aorta of rats[4]. The aortic denudation model performed with a balloon catheter is also commonly used in laboratory rats[5,6]. The intimal hyperplasia model performed in laboratory mice[7] implements ligation of the carotid artery and induces myointimal lesions[8]. In our lab, the abdominal aortic denudation model – to study the development of myointimal hyperplasia – as well as a humanized stent restenosis model[9] are commonly used. This video emphasizes that choosing the proper experimental animal model is crucial for mechanistic or pathophysiologic studies of arterial stenosis.

Myointimal hyperplasia models must be distinguished from atherosclerosis models. For the latter, apolipoprotein E-deficient (ApoE -/-) mice in combination with western diet are commonly used to induce atherosclerotic lesions[10,11,12,13]. Examples for the induction of both of these types of myointimal disease in mice are shown here, along with different model-adapted options to analyze vessel stenosis[14].

Protocol

All animal work should be carried out according to relevant animal care guidelines. Obtain institutional approval for animal work prior to beginning protocol.

1. Myointimal Hyperplasia Model (A)

For the intimal hyperplasia model, purchase C57BL/6J (Stock number 000664, C57BL/6J) mice at the age of 8 weeks weighing approximately 25 g. House mice for these experiments under conventional conditions; feed mice standard mouse chow and provide water *ad libitum*.

1. Mouse preparation: Introduce the mouse to an anesthetic state with isoflurane (2%) utilizing an induction chamber.
    1. Remove the abdominal hair using a hair trimmer, lay the mouse on its back and cover its nose and mouth with the facemask to assure the anesthesia.
    2. With Provo-Iodine, disinfect the abdominal area universally, followed by 80% ethanol, in two cycles.
    3. Assure the absence of reflexes by pinching the hind limb to monitor the sufficient depth of anesthesia.
    4. Separate the skin and muscle along the linea alba in two steps to expose the abdominal organs.
    5. Lay the intestines in a saline moisturized glove. Wrap the glove around the intestines to keep them moist.
    6. Attentively clear away the fatty tissue sheeting the abdominal aorta. Expose the infrarenal aorta down to its bifurcation, taking care not to injure any branch vessels.
    7. First, place a microsurgical clamp on the proximal, infrarenal aorta to shut down the blood flow. Now, apply a second distal clamp next to the aortic bifurcation.
    8. When the blood flow is interrupted, make a small incision into the aorta close to the proximal clamp.
    9. Insert the balloon-tipped catheter, which has been moisturized with 0.9% saline, and advance towards the distal clamp of the aorta.
    10. For endothelial injury, denude the aorta by inflating the balloon carefully and slowly pulling the catheter into the direction of the proximal clamp.
    11. Repeat the denudation maneuver twice.
    12. Remove the catheter and close the aortic incision using 10-0 prolene running sutures.
    13. Carefully open the distal clamp. In case of bleeding at the aortotomy site, close the clamp again, locate the bleeding and place additional interrupted stitches, as necessary.
    14. If no bleeding can be observed, slowly open the proximal clamp.
    15. An aortic pulse should be visible distally from the incision. Now the intestines can be arranged back *in situ*.
    16. Rinse the abdominal cavity utilizing pre-warmed sterile saline.
    17. Close the abdominal wall beginning with the muscle layer using 6-0 prolene running sutures.
    18. Next adapt the skin under the use of 5-0 prolene performing running sutures.
    19. During the time the mouse is still anesthetized, apply 4-5 mg/kg Carprofen via a subcutaneous injection.
    20. Add Metamizol to the drinking water (50 mg Metamizol per 100 ml) for pain control and continue for 3 days post surgery. Monitor animal throughout recovery.

        Note: The observation period for this model is 28 days.

2. Atherosclerosis Model (B)

For the atherosclerosis model, purchase apolipoprotein E-deficient (ApoE -/-) mice (Stock number 002052, B6.129P2-*Apoetm1Unc*/J) at an age of 4 weeks, and then fed for 4-6 months. House mice for these experiments under conventional conditions; feed mice high lipid western diet upon arrival (Harlan Laboratories TD.88137) and provide water *ad libitum* for a duration of 4 to 6 months.

1. Mouse preparation:
    1. Feed the ApoE -/- mice with western diet beginning at 4 weeks of age. Dietary manipulation should begin directly upon arrival.
    2. Maintain western diet throughout duration of experiment.

3. Analysis

1. Myointimal hyperplasia model (A)

    The region of interest in this model is the “lesion area”. Options for analysis include luminal obliteration, I/M ratio (intima/media ratio), maximal myointima thickness, myointimal area, Trichrome staining for fibrosis, and H&E staining for mononuclear cells (as illustrated in **Figures 1A-1C**). According to the scientific question numerous other stainings like Picrosirius red for collagen, immunohistochemistry, and confocal methods can be applied.
2. Atherosclerosis model (B)

    The regions of interest in this model are the aortic valve, aortic arch as well as the descending aorta. Options for analysis: Sudan red staining for lipids and trichrome staining of aortic valve, aortic arch and descending aorta (as illustrated in **Figure 1D**).

    This model also yields a variety of other staining options like for example Oil Red O for neutral lipids, Picrosirius red, immunohistochemistry, and confocal methods.

1. Sudan red staining: Perform the Sudan red staining on freshly harvested aortic tissue.
    1. Remove the diet 6h before harvesting.
    2. Introduce the mouse to an anesthetic state with isoflurane (2%) utilizing an induction chamber. Assure the absence of reflexes by pinching the hind limb to monitor the sufficient depth of anesthesia.
    3. Open the chest; cut off the apex of the heart and perfuse via the left ventricle, first with saline (3 ml), followed by 4% PFA (3 ml) through the aortic root to fix the aorta.
    4. Free the aorta from the root to its bifurcation. Try to remove fatty tissue as much as possible.
    5. Cut off the heart and the aortic root, and store them in 4% PFA for histology.
    6. For histology the aortic root has to carefully be placed in the paraffin block in an upright position to allow the cutting of the aortic root generating slides that display all 3 valvular leaflets.
    7. Cut one end of the aortic tree and fix it onto the silicone layer with fine pins. Open the aorta longitudinally and fix the aortic tissue.
    8. Rinse the tissue with 50% ethanol. Immerse the tissue with Sudan III staining solution for about 15 min.
    9. Rinse lightly with 50% ethanol to remove the excess Sudan III. (Note: 50% ethanol may wash the dye away.) Rinse with dH_2O, and take photos for further analysis.

Representative Results

Figure 1 shows different analysis options and the induced disease state for both models. For the intimal hyperplasia model, the analysis of representative cross sections in trichrome staining is shown. From these cross sections, results like I/M ratios, maximum plaque thickness, maximum intimal thickness in the lumen, luminal obliteration in percent, as well as plaque area can be measured and calculated.

To evaluate the outcome of the atherosclerosis model, all methods named above can be used. In addition, the quantitative plaque area in the abdominal aorta, the extent of plaque formation at the aortic valve, the aortic arch, and the descending aorta can be measured via trichrome staining.

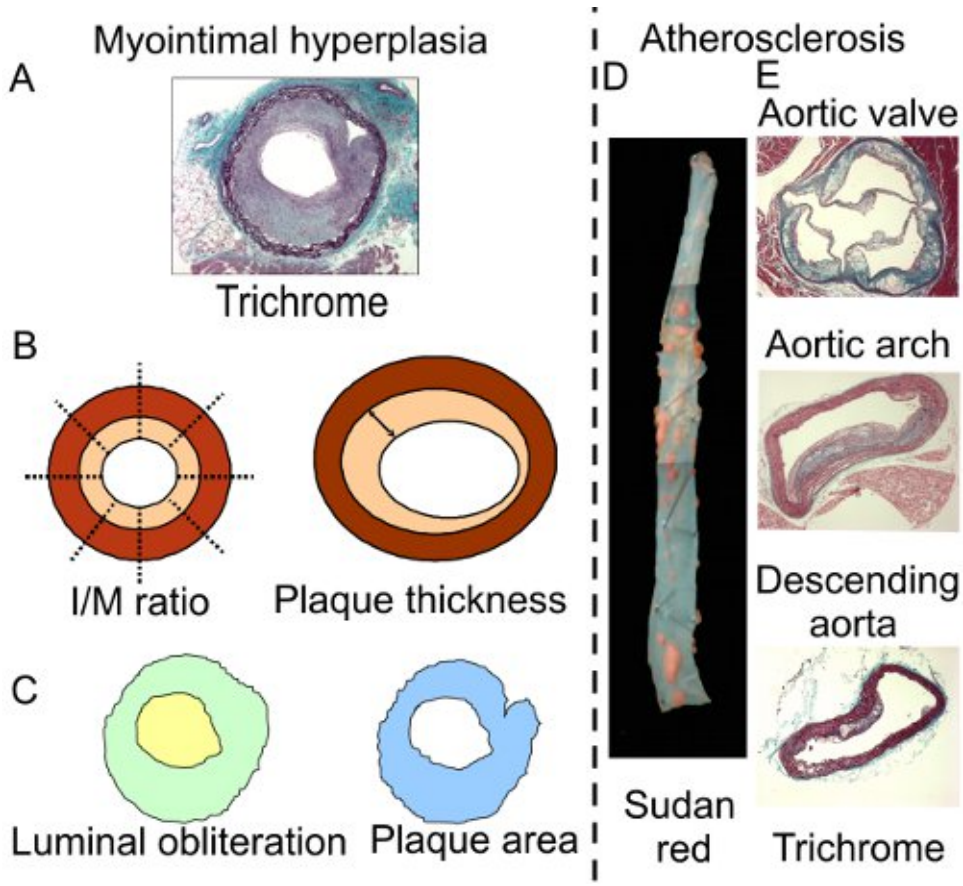

Figure 1. Denuded mouse aortas are harvested, paraffin embedded, and a representative cross section is shown in trichrome staining **(A)**. I/M ratios are calculated on trichrome stained sections by measuring intima and media thickness using computer analysis followed by calculation of the ratio **(B)**. Maximum plaque thickness is measured as the maximum intimal thickness in the lumen by computer morphometry **(B)**. Luminal obliteration (in percent) is determined by subtracting the new inner lumen from the former lumen, divided by the former lumen, multiplied by 100% **(C)**. The plaque area is assessed by subtracting the new inner lumen from the former lumen **(C)**. The descending aorta, opened longitudinally with lipophilic structures and stained with Sudan red is shown **(D)**. To determine the quantitative plaque area, Sudan red stained lesions as well as the total aortic area, are measured and analyzed using image analysis software. Cross sections of paraffin embedded samples of the aortic valve, the aortic arch, and the descending aorta in trichrome are depicted **(E)**. Luminal obliteration, plaque area, and maximum plaque thickness are calculated as described above. Please click here to view a larger version of this figure.

Discussion

Although both diseases result in similar symptoms in the patient, the underlying mechanisms of plaque development and therefore the treatment approaches are very different[2]. In different forms of arterial stenosis the clinical findings in the patients as well as the timeframe of plaque development depend on the underlying mechanism.

Depending on the clinical findings in the patient, different methods of sample analysis must be performed[5,15]. It is necessary to adapt the analysis to the characteristic findings in the disease that is being mimicked by the chosen model[14]. Vice versa, depending on the disease being studied, the most feasible and appropriate animal model should be chosen. Moreover, it is crucial that new drugs should mechanistically match the model.

Both models introduced in this publication are quick and easy to perform and highly reproducible. There are some major differences between the models. For example, the observation time in the myointimal hyperplasia model is 28 days[16], while atherosclerotic plaque development needs four to six months[17].

Another major difference is the applicability to different mouse strains. The atherosclerosis model relies on the ApoE -/- or LDL -/- in mice and can therefore only be performed in mice carrying this specific knockout. To create mice carrying both the ApoE -/- and another desired knockout is very time consuming. The myointimal hyperplasia inducing balloon injury surgery can be performed in any mouse. The myointimal hyperplasia model yields more potential to modify the mouse strain of interest, while the atherosclerotic model faces a strict limitation to mice carrying the ApoE -/-.

The intensity of balloon denudation is crucial for the outcome of the myointimal hyperplasia lesions. If the catheter injury is performed too aggressively, aneurysm formation might occur; if the denudation is performed too gently, plaque development might be too weak to see differences between groups. In this procedure it is helpful if all surgeries within one study are performed by the same person. The advantage of

the balloon injury towards other techniques that require the introduction of stiff or sharp obstacles like wires, is the flexibility as well as the option to adjust the size and pressure of the balloon via the inflation of the balloon-tipped catheter. Furthermore the uninflated balloon-tipped catheter is thin and can be inserted into the aorta via a relatively small incision compared to thicker, more rigid denudation tools. For the atherosclerotic plaque formation model it can be crucial to start feeding the western diet at a young age of the mice because that can have a large impact on the severity of the lesions. Since lesions in both introduced models develop in both female and male mice, the gender can be adapted to the requirements of the experiment.

Disclosures

The authors have nothing to disclose.

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