Role of Sympathetic Denervation in the Development of Vascular Calcification

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Introduction

Current Knowledge: Vascular calcification is an active process driven by transdifferentiation of vascular smooth muscle cells (VSMCs) from contractile to osteo/chondrogenic phenotypes. VSMCs are innervated in healthy conditions and rely on many types of signaling to maintain phenotype.

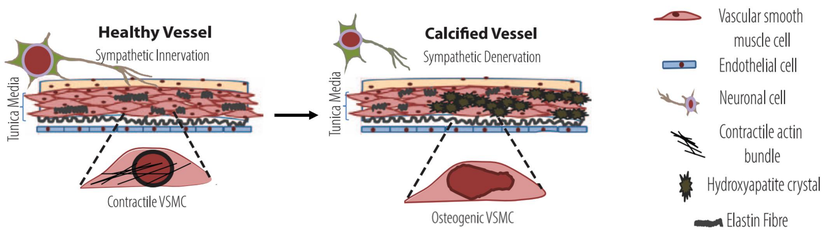

Figure 1. VSMC phenotype changes from contractile to osteogenic in calcification pathogenesis. Proposed relationship with denervation is also displayed. Key pictured to the right. Image modified from Ho et al. [1].

Problem: Calcification pathogenesis is not fully characterized, making treatment development difficult.

Goal: Develop a sympathetic denervation animal model to study the impact of disrupted nerve signals on calcification.

Hypothesis: Sympathetic denervation will disrupt signals to VSMCs that help maintain a contractile phenotype, leading to osteogenic transdifferentiation and calcification.

Methods

Animals*: 4 to 14-week-old male BALB/c mice (n = 8 total)
Artery: Femoral artery
Neurotoxin: 6-hydroxydopamine (6-OHDA)

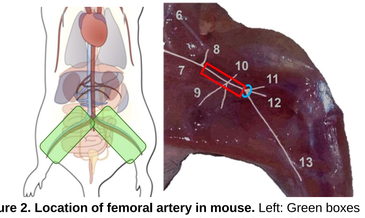

Figure 2. Location of femoral artery in mouse. Left: Green boxes show location relative to the rest of the body. Right: Red box outlines treatment area. Blue ellipse demonstrates location of suture at harvest.

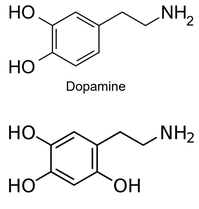

Figure 3. Structure of dopamine and 6-OHDA. Specificity of 6-OHDA relies on its structural similarity to dopamine to bind dopamine transporter in sympathetic neurons. Once inside the cell, 6-OHDA creates oxidative stress [2].

Procedure: Artery was surgically dissected away from the femoral nerve bundle and soaked in 6-OHDA. Contralateral control artery was treated with buffer vehicle solution.

Group	1 Week	2 Weeks	3 Weeks
Injury	n = 2	n = 3	n = 3
Control	n = 2	n = 3	n = 3

Analysis: Hematoxylin & Eosin (H&E) stain and immunofluorescence (IF) for tyrosine hydroxylase (TH) and α-smooth muscle actin (α-SMA)

*All animal work approved by Northwestern IACUC

Results

- Artery was successfully separated from the nerve bundle
- 100% survival rate
- Optimization of harvest, paraffin embedding, and sectioning technique completed

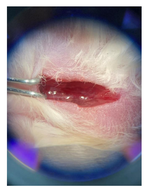

Figure 4. Surgical set up for injury limb. After artery and vein were separated from the nerve bundle, 6-OHDA (or buffer for control) was added drop-wise into the incision until the artery was fully submerged as shown.

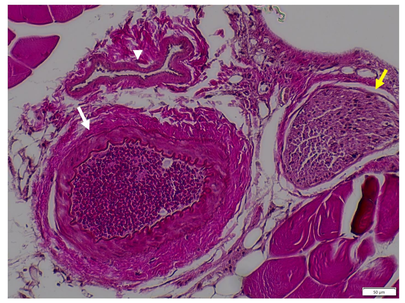

Figure 5. Cross-section of artery, vein, and nerve bundle stained with H&E. Artery labeled with white arrow, vein with white arrowhead, and nerve bundle with yellow arrow. Scale bar: 50 μm.

IF Key	Abbreviation	Cell Type Labelled	Color
Tyrosine Hydroxylase	TH	Sympathetic Neuron	Green
α-Smooth Muscle Actin	αSMA	VSMC	Red
Hoechst Nuclear Stain	N/A	All Nuclear Cells	Blue

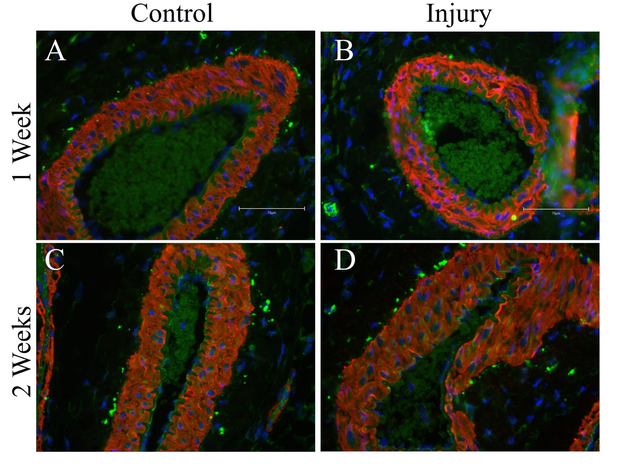

Figure 6. IF images of control and injured arteries from one and two weeks after surgery. (A-B) The injury limb showed fewer sites of innervation than the control at one week, shown by the reduction in positive TH stain. (C-D) At two weeks, there is no noticeable difference between positive TH staining between the injury and control limbs. Scale bars: 75 μm.

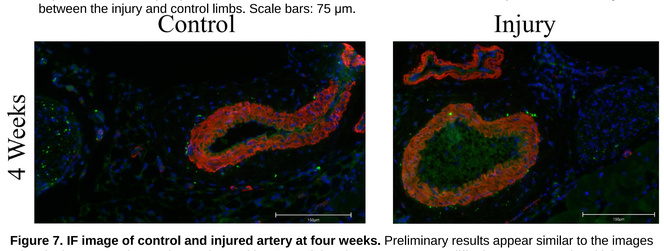

Figure 7. IF image of control and injured artery at four weeks. Preliminary results appear similar to the images taken in the two-week samples as the positive TH staining does not appear to differ between control and injury groups. Scale bars: 150 μm.

Discussion

Initial Denervation

Denervation of murine femoral arteries with 6-OHDA was successfully achieved by one week.

Sympathetic Nerve Presence

Preliminary data from the animals sacrificed at two weeks shows no obvious reduction in innervation in the injured tissue compared to the control, possibly signifying nerve regeneration or axonal growth.

Possible Causes

Nerve regeneration or growth has been shown to occur in rabbits four weeks after treatment with 6-OHDA [3].

Artemin is one factor that may play a role, as it is known to promote migration, proliferation, and differentiation of sympathetic neuron precursors [4].

Figure 8. Artemin Structure. This member of the GDNF family has been shown to play an important role in axonal migration [5] and peripheral neuronal maintenance [4] and may support neuronal survival and regrowth after denervation by 6-OHDA.

Conclusions

1. 6-OHDA is a viable sympathetic neurotoxin for mouse femoral artery denervation for up to one week.
2. Denervation is reversed at two and four weeks after treatment with 6-OHDA.
3. Further investigation is necessary to determine if denervation period can be prolonged and if this injury can cause calcification.

Future Directions

Analysis			
Histology	von Kossa (Calcium)	Masson's Trichrome (ECM)	Verhoeff-Van Gieson (Elastin)
Immunofluorescence	Osteocalcin	Artemin	General Neuronal Markers
Quantitative Analysis (ImageJ)	Wall Thickness		

Future Experiments

- Artemin *in vitro* studies (siRNA)
- Surgical Adjustments
  - 6-OHDA application time
  - 6-OHDA reapplication
  - Include Female Animals

If development of vascular calcification is successful in this model, nerve regeneration or stimulation strategies may be viable targets for therapeutic intervention.

References

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