Development of Femoral Artery Denervation Models for Vascular Remodeling

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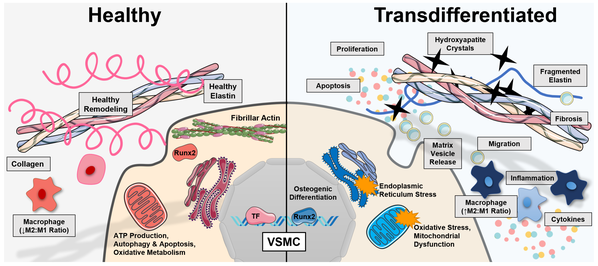
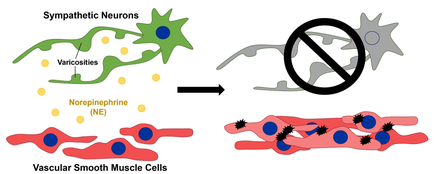
Introduction

Background: Healthy arteries are innervated by the sympathetic nervous system to not only regulate vascular smooth muscle cell (**VSMC**) contractility and tension, but also to regulate arterial maturation and structure. [1] Tissue innervation is a critical component for successful regeneration in any transplanted tissue. [2] However, little is known regarding vascular remodeling due to sympathetic nerve degeneration or injury. One potential consequence of sympathetic denervation is VSMC transdifferentiation. VSMCs can switch from a contractile phenotype to a synthetic (proliferative) [3] or osteo-chondrogenic (bone-like) [4] phenotype. Proliferation and migration can thicken the vessel and restrict blood flow, while osteogenic cells can deposit hydroxyapatite crystals into the artery wall.



Hypothesis: Denervation of arteries will initiate a sequence of events that will lead to the phenotypic changes of VSMC and vascular remodeling.

Goal: Elucidate the relationship between sympathetic innervation and vascular pathogenesis by creating a novel mouse model of arterial denervation.



HYPOTHESIS: Sympathetic denervation of the femoral artery will lead to transdifferentiation of arterial VSMCs and pathological remodeling of the arterial structure

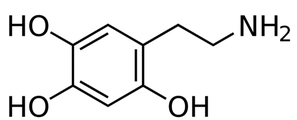
Methods

Animals*: Male BALB/c mice

Procedures:

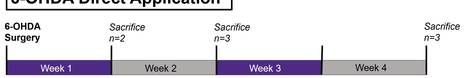
A) Femoral artery surgically exposed for direct application of neurotoxin 6-hydroxydopamine (**6-OHDA**) or buffer control

B) 6-OHDA subcutaneously injected on a weekly basis in region of femoral artery for four weeks with buffer injection as control

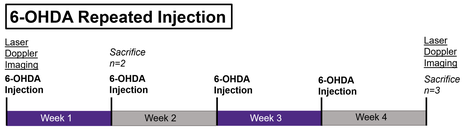


6-hydroxydopamine

6-OHDA Direct Application



6-OHDA Repeated Injection



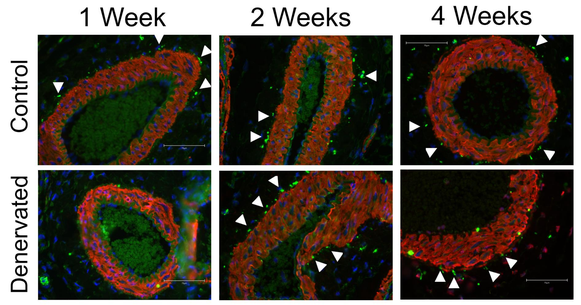
Analysis:

- Innervation and extracellular matrix (**ECM**) remodeling studied with histology and immunohistochemistry
- Blood flow with Laser Doppler Imaging

*All animal work approved by Northwestern IACUC

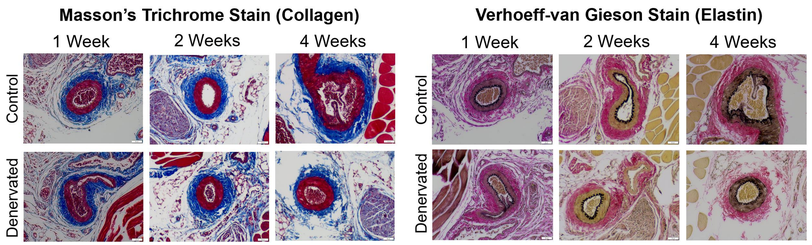
Results

1. Sympathetic denervation of murine femoral arteries can be achieved by direct application of 6-OHDA after open surgery. However, the effect is reversed at two and four weeks after one-time direct application of 6-OHDA, possibly due to nerve regeneration.

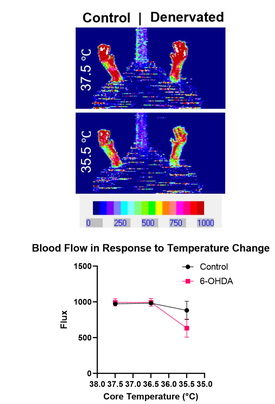


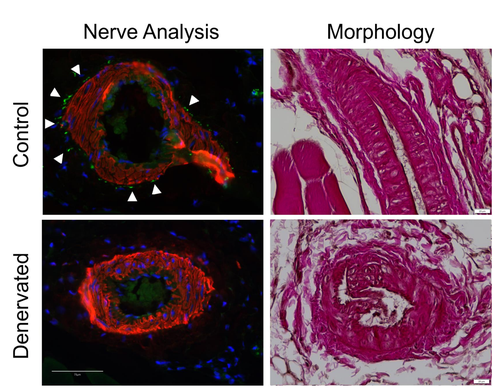
IF Key	Cell Type	Color
Tyrosine Hydroxylase (TH)	Sympathetic Neuron	Green
α-Smooth Muscle Actin (αSMA)	VSMC	Red
Hoechst Nuclear Stain	All Nuclear Cells	Blue

2. One-time direct application of 6-OHDA is insufficient to cause remodeling of arterial ECM.



3. Weekly injection of 6-OHDA leads to sustained denervation and lower blood perfusion at low temperature.





Discussion

Denervation

- Spontaneous sympathetic nerve reappearance at two weeks in otherwise healthy mice is a good sign that nerves can grow back toward arteries when the artery itself stays intact
- Nerve reappearance at two weeks in this model shows that this timeline is possible for regeneration, so future attempts at regeneration can be compared against this
- There are natural mechanisms for nerve regeneration we could seek to replicate for therapies
- Injection is a much more efficient method that allows for repeat administration, and does not affect the control limb
- Histology after four weeks of repeated injections demonstrates denervation of the treated limb and not the control

Remodeling

- If ECM remodeling can be stimulated by denervation, it is a slower process
- If denervation alone cannot stimulate sufficient remodeling, perhaps a combination model with other types of clinically relevant damage could provide more information

Regulation of Blood Flow

- Arterial denervation causes changes in hemodynamics in response to temperature changes, though the specific mechanisms remain unclear
- Other systemic processes such as cardiac output could be influencing the hemodynamics independent from vasoconstriction

Future Directions

Experiments

- Combine denervation with models of endothelial injury or peripheral arterial disease
- Transdifferentiation protein analysis

↑ **Runx2**
Alkaline Phosphatase
Osteocalcin

↓ **α-Smooth Muscle Actin**
Smooth Muscle Myosin
Calponin

IMPACT: If sympathetic denervation causes VSMC transdifferentiation and pathological remodeling, nerve regeneration or stimulation strategies may be viable targets for therapeutic intervention.

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References

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