Impaired vascular reactivity following chronic ischemia in the arteries of the mouse hindlimb

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Introduction
Patients with ischemic disease, such as peripheral artery disease (PAD), demonstrate impaired vascular reactivity and blood flow control. The presence of co-morbidities common in these patients, such as hypercholesterolemia, likely contribute to the impaired vascular reactivity. However, ischemia in the absence of co-morbidities (i.e. in otherwise healthy animal models) is also known to impair vascular reactivity in arteries and reduce collateral-dependent hyperemia. Although impaired vascular reactivity and blood flow control are well described in animal models of peripheral ischemia, the cellular mechanisms of these impairments are poorly understood. Further, little is known about the behavior of individual arteries following chronic ischemia, as most of this literature focuses on arterioles in the lower leg or regional blood flow in the thigh. Therefore, the goal of our work is to determine the impact of chronic ischemia on feed artery reactivity, and to define the cellular mechanism of any observed impairments. The long-term goal of this work is to determine the relative contribution of ischemia to impaired reactivity in patients with ischemic disease.

Mouse Hindlimb Ischemia Models
To determine the impact of ischemia on feed artery vascular reactivity, we utilized an experimental model that involved resection of the femoral artery-vein pair from just upstream of the muscular branch to the distal saphenous, Figure 1. This surgery removes the femoral artery supply to the muscular branch and therefore induces ischemia in the muscular branch artery.

Intravital Microscopy

Figure 1. Medial aspect of the mouse hindlimb. The region of femoral artery-vein resection is indicated with a dashed line.

We measured feed artery diameter using Side-stream Dark Field (SDF) intravital microscopy. SDF collects the reflected light from oblique 530nm pulses. 530nm is the isobestic point for hemoglobin, thus the vasculature appears dark owing to the absorbance of light by red blood cells; the parenchymal and stromal tissue appears light. Our intravital imaging station is shown in Figure 2. Example images of the muscular branch artery (at rest and following functional vasodilation) are shown in Figure 3.

In addition to electrode position, we also examined the impact of pulse frequency, stimulus duration, and stimulus amplitude in developing our functional vasodilation protocol. Increasing stimulus frequency leads to an increase in muscular branch vasodilation, Figure 6. Varying stimulus intensity had a limited affect on diameter in the muscular branch artery, Figure 7.

Ischemia Eliminates Functional Vasodilation in Response to Moderate Muscle Contraction

To examine the impact of ischemia on muscular branch artery diameter, we measured functional vasodilation at day-14 following femoral artery-vein resection surgery, using the following stimulation parameters: 1mA, 200μs, 8Hz, 90sec. These stimulation parameters failed to elicit a functional vasodilation in the ischemic muscular branch arteries, Figure 8.

Ischemia Impairs Vasoconstriction Following Intense Muscle Contraction

Figure 11. Functional vasodilation in the ischemic muscular branch collateral artery following high intensity contraction. Sham operated (black circles) and day-14 ischemic arteries (grey circles).

Summary
• Ischemia eliminates functional vasodilation in response to moderate intensity muscle contraction, Figure 9.
• Functional vasodilation is normal in the ischemic muscular branch artery if a more intense muscle contraction is induced, Figure 10.
• Ischemic muscular branch arteries are refractory to vasoconstricting back down to their resting diameter following the cessation of muscle contraction, Figure 11.

Future Work

The ability of the ischemic muscular branch to vasodilate only in response to intense muscle contraction (Figure 10) and not moderate muscle contraction (Figure 9) suggests that different vasodilation pathways are operational in the ischemic artery, or that the typical pathways are less sensitive to dilating stimuli. Specifically, we hypothesize that endothelial-dependent vasodilation is impaired in the ischemic muscular branch artery while smooth muscle dependent vasodilation is preserved. Therefore, upcoming studies will use superfusion intravital microscopy to examine cell specific vasodilation responses, such as those in Figure 12.