Ischemic Stroke Alters Blood Vessel Branching and Size Distribution in the Distal Femur

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INTRODUCTION: Stroke patients have higher fracture rates and lose bone mass more quickly than healthy individuals of similar age[1]. Vascular elasticity, a measure of vascular health, is correlated with BMD in the radius in human stroke patients, suggesting post-stroke bone loss is concurrent with declining vascular health[2]. We previously showed that ischemic stroke in mice resulted in transient decreases in blood perfusion (proximal tibia)[3] and increases in blood vessel volume (distal femur)[4] compared to sham mice, but the impact of stroke on the vascular structure and endothelial composition remain unknown. Specifically, thin arterioles (5-20µm diameter) near the growth plate that express both endomucin and CD31 (Type H cells) have been shown to couple angio- and osteogenesis[5]. The objective of this study was to determine whether the osteovascular structure or type would be altered following stroke.

METHODS: Under IACUC approval at North Carolina State University, 12-wk-old, male C57Bl6/J mice received either a stroke (n=15) or sham (n=12) surgery. Ischemic stroke was induced using the well-established middle cerebral artery occlusion (MCAo) model that closely mimics ischemic stroke in humans[6], using a coated filament inserted for 30 min and then removed to allow reperfusion. For sham, neck incision was left open for 30 min without MCAo. After 4 days recovery, mice were divided into groups for exercise (n=6 'sham ex', n=8 'stroke ex') with moderate treadmill activity (9 m/min, 37 min, 5 d/wk) and sedentary (n=6 'sham sed', n=7 'stroke sed') with stationary treadmill for same duration. After 4 wks recovery, mice were sacrificed, and hindlimb bones removed. Vascular structure in distal femur was measured using a polyoxometalate contrast agent with micro-computed tomography (2-µm voxels)[7]. Blood vessel branching and diameter were quantified ('Analyse Skeleton' command in BoneJ)[8]. Thick section IHC (50 µm) was used to quantify amount of CD31- and endomucin-expressing endothelial cells in the proximal tibia, based on labeled areas (MATLAB, ImageJ)[9]. Group differences were assessed using two-way ANOVAs with Tukey's post-hoc comparisons (α=0.05, R Statistical Computing). Vessel thickness distribution was assessed using a mixed-effects linear model with repeated measures and Tukey's post-hoc comparisons (α=0.05, SAS).

RESULTS: Mean vessel thickness in the distal femur was not affected by stroke (p=0.36, Fig. 1B), although compared to sham, stroke mice had fewer vessels of 6-26 µm in thickness but more vessels of 38-54 µm in thickness (p<0.05, Fig. 1A). Compared to sham, the vascular network of stroke mice had more branches (p=0.001, Fig. 1C). We found no differences in the areal fraction of endomucin+ cells (p=0.36 stroke effect, p=0.52 exercise effect), CD31+ cells (p=0.49 stroke effect, p=0.15 exercise effect), or Type H cells (p=0.50 stroke effect, p=0.14 exercise effect) (Fig. 2). We found no significant differences between the affected (paretic) and unaffected limbs in any of the outcome measures, indicating systemic changes in metrics presented here.

DISCUSSION: Ischemic stroke increased vascular branching and increased the amount of thicker blood vessels in the distal femoral metaphysis after 4 weeks. Osteovascular growth and widening post-stroke may be stimulated by inflammation, which promotes angiogenesis but also stimulates osteoclasts[10] and interferes with Notch signaling - the primary mechanism of Type H cell regulation of bone osteogenesis[5]. In these preliminary data, the amount of Type H cells in the proximal tibial metaphysis was not affected by stroke. However, stroke did decrease the amount of thin blood vessels (5-20µm diameter) in the distal femur, which are found close to sites of osteogenesis and could play a role in detrimental bone changes experienced by stroke patients[5]. Future work will further examine the vascular structure to determine the distance of blood vessels to bone surfaces[7,11].

SIGNIFICANCE: Although human stroke patients lose bone mass rapidly, we observed an increase in the volume, thickness, and branching of the vascular network in the hindlimb bones of mice following ischemic stroke. Further exploring the mechanisms for vascular changes following stroke may inform therapies to mitigate stroke-related fractures in human patients.

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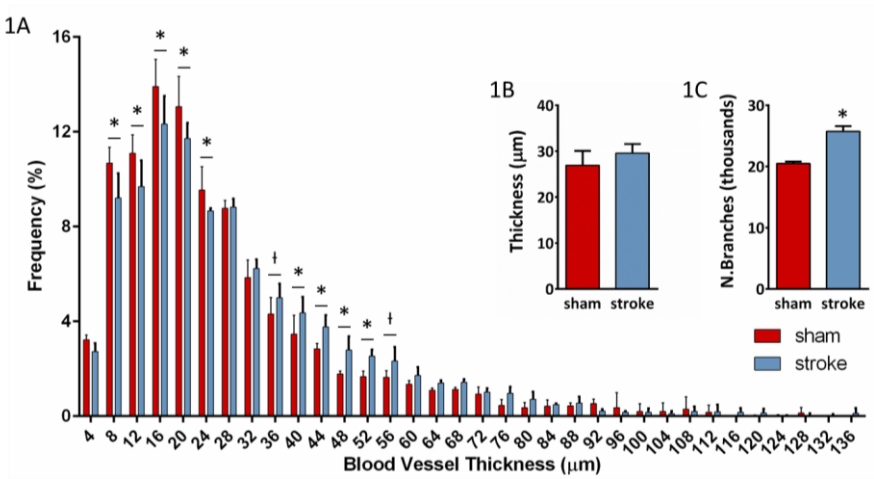

Figure 1: A) Blood vessel thicknesses distribution in distal femur. B) Mean vessel thickness not affected by stroke. C) Increased number of vascular branches in stroke vs. sham. *p<0.05, †p<0.1.

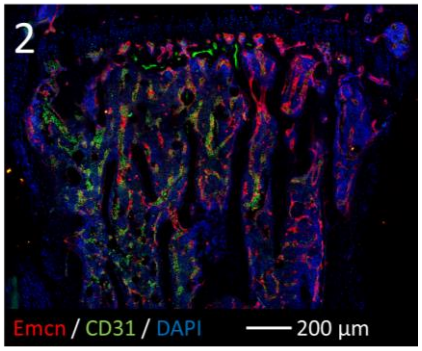

Figure 2: Representative image of thick-section IHC in proximal tibial metaphysis. Most sections had abundant endomucin (red) staining but little CD31 (green).