Examining Changes in Bone Perfusion and Bone-Specific Biomarkers with Ischemic Stroke

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INTRODUCTION: Stroke survivors lose bone mass more quickly and experience fractures at a higher rate than typical aging adults.[1,2] Vascular elasticity is correlated with bone strength in the forearm following stroke,[3] suggesting bone loss post-stroke is concurrent with declining vascular health. We have previously shown changes to vascular structure in bone (*osteovasculature*) and decreased intraosseous perfusion following ischemic stroke in young male mice.[4] However, osteovascular changes have not yet been examined in older mice, which more closely mimic the affected population. Unique serum metabolites are perturbed following acute ischemic stroke, indicating systemic dysregulation of metabolic pathways,[5] but bone-specific metabolites have not yet been identified. The objectives of this study were to determine age-specific changes to osteovasculature post-stroke and identify bone-specific biomarkers unique to stroke, which may elucidate potential mechanisms contributing to stroke-related bone fragility.

METHODS: Under IACUC approval at North Carolina State University, C57Bl6/J mice of two age groups, young (12 wk) and old (27 wk), received either a stroke (n=20) or a sham (n=22) surgery. The middle cerebral artery occlusion (MCAo) model,[6] which closely mimics stroke in humans, was used to induce ischemic stroke by inserting a coated filament for 30 min and then removing to create reperfusion injury. For sham surgery, the neck incision was left open for 30 min without MCAo filament. Neurological and motor assessments (*neuroscores*) were performed on days 1, 2, 3, 4, and 7 post-surgery and weekly thereafter.[7] Tibial perfusion was measured using laser Doppler flowmetry on days 4 and 7 post-surgery and weekly thereafter.[8] After a 4-week recovery, mice were sacrificed, and humeri and tibiae removed. Humeri from stroke and sham groups were bisected longitudinally, embedded in plaster of Paris, and analyzed using infrared matrix-assisted laser desorption electrospray ionization (IR-MALDESI)[9] mass spectrometry imaging (MSI).[10] MSI data was collected across 250-1000 mass-to-charge (*m/z)* range, features were putatively annotated using METASPACE,[11] and ion heatmaps were generated using MSiReader[12] imaging software. Neuroscore comparisons were performed using a repeated measures factorial model with Tukey-Kramer adjustments (α = 0.05, SAS). Tibial perfusion comparisons were made using a mixed hierarchical linear model with Tukey-Kramer adjustments (α = 0.05, SAS).

RESULTS: In both age groups, neuroscores showed pronounced sensorimotor impairments in stroke animals in the first week that then improved during the 4-week recovery. While neuroscores were not significantly different between age groups, day-dependent differences were observed ($p<0.01$). In young mice, tibial perfusion was reduced for the first 2 weeks following stroke but returned to sham levels at Week 3 ($p<0.01$) (Figure 1). In old mice, perfusion was lower in stroke compared to sham only at Week 2 ($p<0.01$). Compared to young mice, old mice (stroke and sham) had higher perfusion throughout the recovery period ($p<0.01$). Preliminary MALDESI-MSI results showed detection of phosphatidic acid (PA) and ceramide (Cer), with corresponding ion heat maps for semi-quantitative analysis of relative intensity and spatial distribution of these small molecules (Figure 2A-B).

DISCUSSION: Neuroscores confirmed sensorimotor function was impaired following stroke and improved over the 4-week recovery period, in agreement with our previous observations.[4] Perfusion levels were higher in old than young mice over the recovery period, perhaps explained by angiogenesis induced by minor, chronic inflammation that is a hallmark of aging.[13] PA and Cer are signaling molecules in the TGFβ/Smad[14] and BMP[15] pathways, respectively, which have different roles throughout the body. Localization of PA and Cer to cortical and cancellous bone regions suggests a bone-specific function, indicative of active sites of bone remodeling. Identification of other bone metabolites and vascular metabolites are underway and will be compared between stroke and sham groups. Future work will examine the effects of stroke on vessel size distribution and proximity to bone in these older mice to see if the osteovascular phenotype is less osteogenic as observed in young mice.[4]

SIGNIFICANCE: Ischemic stroke in an MCAo mouse model induced age-specific changes in tibial intraosseous perfusion and perturbations in bone-specific biomarkers. Deeper understanding of osteovascular and metabolic changes may elucidate treatment targets to mitigate bone fragility post-stroke.

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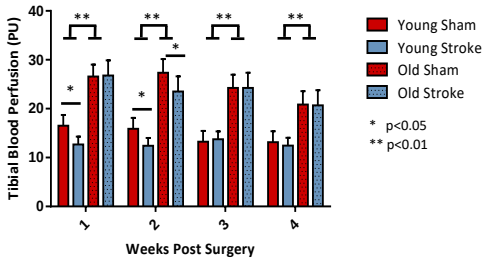

Figure 1. Tibial perfusion for young (12 wk) and old (27 wk) mice over a 4-week recovery period.

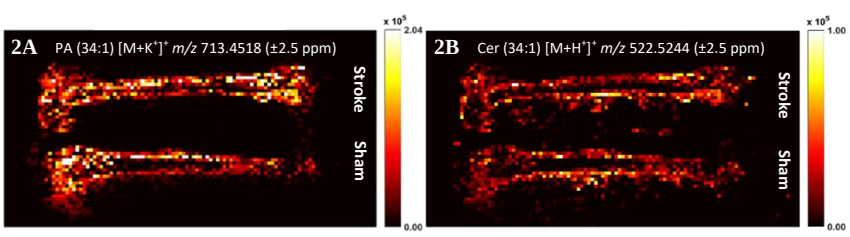

Figure 2. Representative MALDESI-MSI ion heat maps showing distribution of A) phosphatidic acid (PA) and B) ceramide (Cer) within the humerus for stroke and sham groups.