response to a difficult walking environment. We hypothesize that IC will improve stroke survivors' protective stepping response via improvements in muscle activation and motor learning METHODS/STUDY POPULATION: Stroke survivors have an impaired capacity for protective stepping. Decreased paretic muscle activation results in increased reaction time and reduced force generation. Ischemic conditioning (IC) is a vascular stimulus which improves motor performance in chronic stroke. It is performed by delivering transient, intermittent bouts of ischemia to a limb. It has been demonstrated that IC increases muscle activation post-stroke. 9 chronic stroke survivors completed 3 testing sessions and 7 intervention sessions. Participants walked on an instrumented treadmill and were perturbed unilaterally every step at the waist via a cable pulley system. Kinetic and kinematic data were collected. Step width was measured as the difference in position of the heel markers at the instant of heel strike in the frontal plane. RESULTS/ANTICIPATED RESULTS: After one and seven sessions of IC, controls did not alter their responses from baseline testing, but stroke survivors increased their step width by an average of 15% and 23% respectively. DISCUSSION/SIGNIFICANCE: Ischemic conditioning may be a useful intervention to improve stroke survivors' ability to adapt their paretic foot placement in response to lateral perturbations during gait. Interventions which optimize muscle activation and neural adaptation could significantly improve balance post-stroke.

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Pharmacologic Modulation of Endothelial Cell Autophagy During Hypoxic Cold Storage and Reperfusion: Harnessing the Power of 'Self-Eating,' as a Pre-Treatment Strategy for Donor Organs

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OBJECTIVES/GOALS: Donor hearts are transported in cold storage (CS) and undergo ischemia-reperfusion injury (IRI) when transplanted. IRI injures microvascular endothelial cells (EC), heightens the immune response, and has been associated with increased autophagy. We aim to understand the changes in autophagy during CS and IRI and its impact on EC immunogenicity. METHODS/STUDY POPULATION: To study autophagy changes during IRI, immunoblotting for autophagy markers was performed in mouse cardiac ECs (MCECs) lysates. MCECs were in a cold preservation solution in a hypoxic chamber for 6 hours(h) and warm conditions with culture medium for 24 h. MCECs, under standard conditions, served as controls. Secreted interferon-gamma (IFN-γ) levels were quantified via ELISA to study autophagy and EC immunogenicity. MCEC-sensitized CD8+ T-cells were isolated from C57BL/6 spleens and co-cultured with MCECs pre-treated for 16 h with rapamycin or starvation, autophagy inducers, or chloroquine, an autophagy inhibitor under normal or IRI conditions. MCECs without any treatment served as controls. RESULTS/ANTICIPATED RESULTS: To determine autophagy levels in IRI, immunoblotting of MCEC lysates revealed a significant increase ($P<0.01$) in the established autophagy marker, LC3, at early time points post-reperfusion compared to NT conditions, indicating more autophagosome formation during CS and IRI. To assess the role of autophagy in EC immunogenicity, the co-culture experiment revealed that autophagy induction in MCECs under NT and HCS conditions with rapamycin had a 74.9-fold and 51.5-fold reduction of IFN-γ (pg/mL), resepectively, compared to the non-treated controls. In contrast, autophagy inhibition in MCECs with chloroquine resulted in 1.82-fold increase of IFN-γ compared to untreated controls. This suggests a protective role of autophagy in ECs during IRI. DISCUSSION/SIGNIFICANCE: We observed that autophagy may be protective during IRI by mitigating EC immunogenicity. Thus, pharmacologically modulating microvascular EC autophagy in donor hearts prior to transplantation may mitigate insults incurred during CS and IRI.

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Cellular senescence contributes to inflammation and disease progression in an animal model of multiple sclerosis

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OBJECTIVES/GOALS: Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system. MS affects more than two million people worldwide, resulting in physical disability, cognitive impairment, and decreased quality of life. We are investigating the role of senescent cells, a hallmark of aging, in inflammation and disease progression in MS. METHODS/STUDY POPULATION: Female mice on a C57BL/6 background were subjected to the Hooke Laboratory EK-2110 experimental autoimmune encephalomyelitis protocol (EAE; n=10) to induce hind limb paralysis, and matching control mice received no injections (naïve; n=10). Immunofluorescent staining was used to visualize senescence cells and their localization in spinal cord tissue sections from naïve and EAE mice based on antibody detection of cell surface or intracellular proteins. Tissue sections from each group were analyzed in duplicates for each antibody (n=3-4/group). Flow cytometry was performed to immunophenotype the senescence cells using conjugated fluorescent antibodies to cell surface or intracellular proteins (n=5/group). RESULTS/ANTICIPATED RESULTS: Immunostaining demonstrated an increase in the cell senescence markers p16 (15-fold; unpaired t-test, p=0.01; n=3) and p21 (15-fold; unpaired t-test, p=0.003; n=3) in the spinal cord of EAE mice compared to naïve mice. Next, we showed that p16+ and p21+ cells increase with disease progression in the meninges adjacent to the ventral demyelinating lesions (one-way ANOVA, p=0.0009; n=3/group). We further found that 30±9.7% of M2 macrophages, a subset of myeloid cells, express p21 in the spinal cord of EAE mice by using flow cytometry analysis compared to only 5±1.6% in naïve mice (unpaired t-test, p=0.002; n=5/group). DISCUSSION/SIGNIFICANCE: Our findings demonstrate that senescent cells accumulate in the meningeal compartment following EAE induction, suggesting that decreasing senescent cell burden is a promising avenue in delaying disease progression and preventing neuroinflammation in MS.

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